005100
3

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND
TOXIC SUBSTANCES

DATE: 11/22/05

MEMORANDUM

SUBJECT: Aminopyralid Toxicology Data Evaluation Records

PC Code: 005100 ; 005209
DP Barcode: D305670, D305671
TXR No.: 0053657

FROM: Karlyn J. Bailey, Toxicologist
Registration Action Branch 2
Health Effects Division (7509C)

THROUGH: Rick Loranger, Branch Senior Scientist
Registration Action Branch 2
Health Effects Division (7509C)

TO: Joanne Miller, Risk Manager (RM23)
Registration Division (7505C)

Action Requested: The Health Effects Division (HED) was asked to review the submitted toxicology studies conducted with the new chemical, aminopyralid (XDE-750) and its associated triisopropanolamine salt (XDE-750 TIPA); a joint review was done with the the Pest Management Regulatory Agency (PMRA), Canada. The primary toxicology review was performed by PMRA and the the secondary review by HED. The Data Evaluation Records (DERs) are attached for the MRIDs listed in Table 1. The acute toxicity studies were reviewed by the Registration Division (RD).

Background: Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The proposed use is on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, campgrounds, electric utility rights-of way, forestry,

woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

Table 1. List of *Aminopyralid* Toxicology Studies

Study Type	MRID #	Studies included in review	Comments
870.3100 Oral subchronic toxicity-rat	46235621	N/A	New DER
870.3100 Oral subchronic toxicity-rat	46235622	N/A	New DER
870.3100 Oral subchronic toxicity-rat	46235625	N/A	New DER
870.3100 Oral subchronic toxicity-mouse	46235618	N/A	New DER
870.3100 Oral subchronic toxicity-mouse	46235624	N/A	New DER
870.3150 Oral subchronic toxicity-dog	46235623	N/A	New DER
870.3150 Oral subchronic toxicity-dog	46235620	N/A	New DER
870.3200 28 Day Dermal	46235626	N/A	New DER
870.3700 Developmental toxicity-rat	46235631	N/A	New DER
870.3700 Developmental toxicity-rat	46235629	46235633	New DER
870.3700 Developmental toxicity-rabbit	46235632	46284901	New DER
870.3700 Developmental toxicity-rabbit	46235630	46235634	New DER
870.3800 2-Generation Reproduction	46235635	N/A	New DER
870.4100 Chronic Dog	46235627	N/A	New DER
870.4200 Carcinogenicity	46235628	N/A	New DER
870.4300 Combined Chronic/Carcinogenicity	46235615	N/A	New DER
870.5100 Bacterial Gene Mutation	46235636	N/A	New DER
870.5100 Bacterial Gene Mutation	46235637	N/A	New DER
870.5300 Mammalian Gene Mutation	46235801	N/A	New DER
870.5300 Mammalian Gene Mutation	46235804	N/A	New DER
870.5375 Structural Chromosome Aberration	46235802	N/A	New DER
870.5375 Structural Chromosome Aberration	46235803	N/A	New DER
870.5395 Mammalian Erythrocyte Micronucleus	46235805	N/A	New DER
870.5395 Mammalian Erythrocyte Micronucleus	46235806	N/A	New DER
870.6200 Acute Neurotoxicity	46235616	N/A	New DER

870.6200 Chronic Neurotoxicity	46235617	N/A	New DER
870.7485 General Metabolism	46235807	N/A	New DER
NonGuideline- Dissociation and Metabolism	46235833	N/A	New DER

005100

Aminopyralid (DE750) technical / DOW aminopyralid [AMD] / PMRA Sub. No. 2004-0789 — ~ PROTECTED ~ — Rat chronic/oncogenicity / 1 DACO 4.4.4 / OECD IIA 5.5.4

PMRA Primary Reviewer: Steve Wong, Ph.D.
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: [signature]
Date: Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)
Signature: [signature]
Date: 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity and oncogenicity - rat; OPPTS 870.4100; 870.6200; OECD 453.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750

SYNONYMS: aminopyralid; 4-amino-3,6-dichloro-picolinic acid

CITATION: Johnson, KA, and MD Dryzga, March 4, 2004. XDE-750: Two year dietary chronic toxicity/ oncogenicity and chronic neurotoxicity study in Fischer 344 rats. Laboratory project ID 011049, The Dow Chemical Company, Midland, MI, USA. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

In a combined chronic toxicity, oncogenicity, and neurotoxicity study (MRID 46235615), XDE-750 (94.5 % purity) was administered in the diet to groups of Fischer 344 rats, 65/sex/group, at 0, 5, 50, 500, or 1000 mg/kg bw/d. 10/rats/sex/group were used for neurotoxicity assessment; 5 of these rats/sex/group were sacrificed at 12 month for gross and histopathologic examinations of the nervous tissues. Also at 12 months, 10 rats/sex/ group were necropsied for chronic toxicity examination. The remaining animals were maintained on their respective diets for up to 24 months.

All rats were observed daily for mortality and clinical signs of toxicity. They were palpated monthly from months 12 to 24 for tissue masses. Body weight and food consumption were recorded. Hematology, clinical chemistry, and urinalysis were carried out at 3, 6, 12, 18, and 24 months. Ophthalmic examination was conducted prior to dosing and at the end of the study period. At terminal sacrifice, weights of selected organs were recorded, and the animals were subjected to gross and histopathological examination. For rats in the neurotoxicity groups, the following additional parameters were measured: motor activity and functional observatory battery (FOB) at pre-exposure, end of first month, the subsequently at months 3, 6, 9, and 12.

No treatment-related or toxicologically significant effects were observed on clinical signs, mortality, ophthalmoscopy, hematology, or clinical chemistry. The numbers of male and female rats (based on the main groups of 50/group) that survived to study termination were: ♂ = 31, 36, 33, 33, 33; ♀ = 41, 38, 39, 38, 39 at 0, 5, 50, 500, or 1000 mg/kg bw/d, respectively.

Treatment induced toxicity included lower body weight and body-weight gain in both males and females at 1000 mg/kg bw/d. Body weight of males was also slightly depressed at 500 mg/kg bw/d. Food consumption was increased for high-dose male rats. Urinalysis changes were observed at ≥500 mg/kg bw/d. The changes included slightly increased urine volume and decreased specific gravity and

4

pH. Urinalysis changes were unaccompanied by renal histopathologic effects, and they were considered adaptative changes upon fluid homeostasis secondary to the cecal effects and renal excretion of XDE-750. The only target organ was the cecum, which was grossly enlarged. Cecal weights (with ingesta) were increased about four-fold in males and three-fold in females given 1000 mg/kg bw/d for 12 months, but the degree of increase was slightly less after 24 months. Very slight hyperplasia of the cecal mucosa was noted, but this was also less apparent after 24 months than at 12 months. No effects were attributed to XDE-750 at either 5 or 50 mg/kg bw/d. There were no tumors related to XDE-750 ingestion. Based on the body-weight effect, 50 and 500 mg/kg bw/d were considered a no-observed-adverse-effect level (NOAEL) for males and females, respectively. The LOAELs for systemic toxicity for the males and females were 500 and 1000 mg/kg bw/d, respectively. XDE-750 was not carcinogenic to Fischer 344 rats under the conditions of this study.

This study is acceptable and satisfies the data requirement for a chronic toxicity/oncogenicity study in the rat [DACO 4.4.1].

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and data confidentiality statements were provided.

This study was conducted in accordance with the following test guidelines:
1. OECD Guidelines for Testing of Chemicals - Method 453, May, 1981; 424, July 21, 1997
2. US EPA OPPTS 870.4300, 870.6200, August, 1998.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test material:** XDE-750 (4-amino-3,6-dichloro-picolinic acid
Description: tan powder
Lot/Batch #: F0031-143, TSN102319
Purity: 94.5 % a.i.
Compound stability: no information

2 **Test animals:**

Species:	rat	
Strain:	Fischer 344	
Age/weight at study initiation:	7-wk old; ♂ = 119.2-175.3) g, ♀ = 99.1-130.4) g	
Source:	Charles River Labs Inc, Raleigh, NC, USA	
Housing:	2-3 per stainless steel cage	
Diet:	LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO, USA) *ad libitum*	
Water:	municipal water *ad libitum*	
Environmental conditions:	**Temperature:**	20.4-24.7 °C
	Humidity:	40-69 %
	Air changes:	12-15 exchanges per hour
	Photoperiod:	12 h dark/ 12h light (0600-1800 h)
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In life dates** - Start: August 14, 2001 End: August 25, 2003

2. **Animal Assignment/Dose Levels:** Rats were assigned randomly to test groups noted in Table 1.

Table 1: Study design

XDE-750 in diet ppm	XDE-750 intake mg/kg bw/d		Main groups		12-month sacrifice		neurotoxicity assessment (12-month sacrifice)	
	♂	♀	♂	♀	♂	♀	♂	♀
0	0	0	50	50	10	10	10 (5)	10
5	5.1±0.3	5.1±0.2	50	50	10	10	10 (5)	10
50	50.5±3.4	51.2±2.2	50	50	10	10	10 (5)	10
500	505±32	507±21	50	50	10	10	10 (5)	10
1000	1001±68	1018±44	50	50	10	10	10 (5)	10

3. **Dose Selection:**

Selection of doses was based on the findings of short-term dietary toxicity studies (4- and 13-week). For both studies, male and female Fischer rats given XR-750 at 0, 10, 100, 500, or 1000 mg/kg bw/d did not show any systemic toxicity at ≤100 mg/kg bw/d. The only treatment-related finding was increased size and weight of the cecum at ≥500 mg/kg bw/d. Histopathologically, there was very slight epithelial hyperplasia of the cecum and ileum in male rats dosed at 1000 mg/kg bw/d for 13 weeks.

4. **Diet preparation and analysis:**

The test substance was pre-mixed periodically based on stability data. Diets were prepared by serially diluting the pre-mix with ground feed. Subsequently, the concentrations of the test material in the feed were adjusted weekly for the first 13 weeks, and then at 4-week intervals, based on the most recent body-weight and food consumption data. To verify stability, homogeneity, and concentration, liquid chromatography-mass spectrometry (LC-MS) was used. Homogeneity and concentration analyses were carried out at months 0, 4, 8, 12, 18, and 24. Stability of the test material in the feed was demonstrated in the previous 4-week dietary toxicity study. Additional stability of the 7% pre-mix was conducted out to 55 days.

Results -

Stability analysis:
A stability test was conducted with the control and 7% pre-mix after 87 days. All control diet showed below the limit of quantitation of the test material. The 7% pre-mixes showed stability values of 90, 102, and 96.5% of the initial concentration after 41, 54, and 84 days of storage, respectively.

Homogeneity and concentration analyses:
Homogeneity tests were conducted with the test diet preparations at 5 and 1000 mg/kg bw/d on 9 occasions as well as with the 7% pre-mix and the 50 mg/kg bw/d preparation on one occasion. The analytical values were usually within 10% of the targets, with the exception of one 5 mg/kg bw/d preparation.

Concentration analyses were carried out with test diets prepared on 9-10 occasions. The analytical concentrations varied from 90 to 117 of the target concentrations, with the exceptions of 2 preparations which were 86.4 and 86.9 % of the target concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual concentrations was acceptable.

5.. Statistics -

Means and standard deviations were calculated for all continuous data. Body weights, food intake, organ weights, urine volume, urine specific gravity, clinical chemistry, coagulation, and appropriate hematological data were evaluated by Barlett's test for equality of variances. Based on the outcome of the Barlett's test, exploratory data analyses were performed by a parametric or non-parametric analysis of variance (ANOVA). If the ANOVA was significant at $\alpha = 0.05$, it was followed respectively by Dunnett's test or the Wilcoxin Rank-Sum test. DCO incidence scores were statistically analyzed by a z-test of proportions comparing each treatment group to the control group. Descriptive statistics were reported fo body weight gains, feed efficiency, RBC indices, and differential WBC counts. For data from the control and high-dose groups, statistical analyses consisted of the pair-wise comparisons using the Chi-square test. Rare tumors, those with a background incidence of less than or equal to 1%, were considered significant in the Chi-square test at $\alpha = 0.10$, two-sided.

C. METHODS:

Prior to the start of dosing, the rats were evaluated to determine the general health status and acceptability. The rats were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. The rats were fed diets containing XDE-750 at 0, 5, 50, 500, or 1000 mg/kg bw/d

1. Observations:

Animals were inspected at least once daily for signs of toxicity, mortality, and moribundity. Moribund animals not expected to survive until the next observation period were humanely killed. Any animal found dead was necropsied as soon as was practical. A complete detailed clinical observation was conducted prior to dosing, monthly for 12 months, and then at 15, 18, and 24 months on the first 10 survivors from the main oncogenicity groups. Palpable tumor examination was carried out monthly from 12 to 24 months.

2. Body weight

Body weight of individual animals was measured prior to start of the study, at weekly intervals for the first 13 weeks, and at 4-week intervals thereafter.

3. Food consumption and compound intake:

Food consumption was determined prior to start of the study, at weekly intervals for the first 13 weeks, and at 4-week intervals thereafter. The feed containers were weighed at the start and end of a measurement cycle, and the feed consumption was calculated using the following equation:

$$\text{food consumption (g/d)} = \frac{\text{(initial weight of feed container - final weight of feed container)}}{\text{(\# of days in measurement cycle) (\# of animals per cage)}}$$

Test material intake was calculated using the following equation:

Substance intake = $\frac{\text{(feed consumption, g/d) x (\% of test material in feed/100)(FC}_A \text{ x C) x 1000 mg/g}}{\text{(Current bw + previous bw)/2, g) / 1000 g/kg}}$

4. Ophthalmoscopic examination:

All eyes were examined by a veterinarian prior to dosing and at the end of the study period.

5. Hematology and clinical chemistry:

Hematology and clinical chemistry were carried out at 3, 6, 12, 18, and 24 months. Blood samples from 10 rats/sex/group were collected from the orbital sinus of fasted animals anesthetized with CO_2.

a. Hematology - The parameters evaluated are x-marked in the following table.

x	Hematocrit*	x	Leukocyte differential count*	x	Blood clotting Prothrombin time*
x	Hemoglobin*	x	Mean corpuscular Hb (MCH)		Reticulocyte count
x	Leukocyte count*	x	Mean corpuscular Hb concentration (MCHC)		
x	Erythrocyte count*				
x	Platelet count*	x	Mean corpuscular volume (MCV)		
* Required for chronic studies based on Subdivision F Guidelines.					

b. Clinical chemistry - The parameters evaluated are x-marked in the following table.

Electrolytes		**Other**			
x	Calcium*	x	Albumin*	x	Total protein *
x	Chloride*	x	Creatinine*	x	Total bilirubin
	Magnesium	x	Blood urea nitrogen*	x	Glucose*
x	Phosphorus*	x	Total cholesterol	x	Globulins
x	Potassium*		Triglycerides		Serum protein electrophores
x	Sodium*				
Enzymes					
x	Alkaline phosphatase	x	Serum alanine amino-transferase (SGPT)*	x	Serum aspartate amino-transferase (SGOT)*
* Required for chronic portion of combined chronic and oncogenicity studies					

6. Urinalysis

Urinalysis was conducted at 3, 6, 12, 18, and 24 months. Urine was collected from 10 non-fasted rats/sex/group. The urine samples were pooled from each group. The following parameters (X) were examined.

x	Appearance*	x	Glucose*	x	Sediment (microscopic)*
x	Volume*	x	Protein*	x	Urobilinogen
x	Specific gravity*	x	Bilirubin*	x	Ketones*
x	pH	x	Blood*		Nitrate
* Required for chronic portion of combined chronic and oncogenicity studies					

7. Sacrifice and pathology

At the end of the specific study phases, the animals were sacrificed by CO_2 anesthesia, then

4

necropsied and assessed by gross pathology. The animals that died intercurrently or were sacrificed in a moribund state were necropsied as soon as possible after death and assessed by gross pathology. The weight of the fasted animals were recorded at sacrifice. The checked (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	Digestive system		Cardiovascular		Neurologic		Respiratory
	Tongue	x	Aorta*	xx	Brain*	x	Trachea*
x	Salivary glands*	xx	Heart*	x	Peripheral nerve*	x	Lung*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*		Nose
x	Stomach*	x	Lymph nodes*	x	Pituitary*		Pharynx
x	Duodenum*	xx	Spleen*	x	Eyes (optic nerve)*		Larynx
x	Jejunum*	x	Thymus*				
x	Ileum*				Urogenital		Other
xx	Cecum*		Glandular	xx	Kidneys*	x	Bone (sternum only)*
x	Colon*	xx	Adrenal gland*		Urinary bladder*	x	Skeletal muscle*
x	Rectum*		Lacrimal gland	xx	Testes**	x	Skin*
xx	Liver**	x	Mammary gland	xx	Epididymides	x	All gross lesions and masses*
	Gall bladder*	x	Parathyroids***	x	Prostate		
x	Pancreas*	x	Thyroids***	x	Seminal vesicle		
				xx	Ovaries**		
				xx	Uterus*		

* Required for carcinogenicity studies based on Subdivision F Guidelines.
* Organ weight required in chronic studies.
** Organ weight required for non-rodent studies.

II. RESULTS

A. Observations

1. Clinical signs of toxicity -

All in-life observations were considered to be spontaneous changes unrelated to administration of XDE-750.

During the first year, all observations noted were interpreted as unrelated to treatment. Cited observations, except for periocular soiling, were found in only 1-2 rats/sex/dose group and/or there was no pattern suggesting an increased occurrence in relationship to the dose level of XDE-750. Although up to three treated males and five female rats were observed with periocular soiling, the findings were sporadic and lacked a dose response.

During the second year of the study, there were greater numbers and variety of observations than noted through the first year, but this was expected due to geriatric diseases as these rats neared the end of their life span. In the second year, increased numbers of rats were observed with periocular soiling, cloudy eyes, preputial or clitoral gland swelling, dermatitis, or papules of the skin or tail. However, these findings were sporadic and/or lacked a dose response and were considered spontaneous changes unrelated to XDE-750.

Most palpable masses were neoplasms originating from either the skin and subcutis or associated subcutaneous organs including mammary gland, clitoral or preputial gland, or auditory sebaceous gland. There was no pattern suggestive of an effect of XDE-750 for any of the final diagnoses of palpable masses.

2. **Mortality** - There were no treatment-related effects. The mortality data are presented in Table 3.

As is typical for the Fischer 344 rat, there was low mortality for the first 16-18 months of the study, after which mortality increased in all dose groups. Three high-dose males died during the first 12 months, while there was no female deaths during this period. Over the course of the study, 19, 14, 17, 17, and 17 male rats of the main groups died spontaneously or were humanely sacrificed due to moribund condition from the 0, 5, 50, 500, or 1000 mg/kg bw/d dose groups, respectively. For female rats in the main groups, 9, 12, 11, 12, and 11 rats died or were humanely killed from the 0, 5, 50, 500, or 1000 mg/kg bw/d dose groups, respectively.

Table 3: Mortality data of main groups

	mg/kg bw/d	0	5	50	500	1000
Main groups (24 months)	♂ (N = 50/group)	19	14	17	17	17
	♀ (N = 50/group)	9	12	11	12	11

B. Body weight - Representative mean body weights of the main group animals are presented in Table 4.

Table 4: Mean body weights, g

mg/kg bw/d	0	5	50	500	1000
	♂ (N=65/group unless stated otherwise by a number after /)				
day 0	150.5±10.2	147.8±11.5	147.9±10.4	149.5±10.4	151.3±10.8
91	334.3±19.8	330.1±18.2	328.1±18.2	328.9±18.3	325.9±17.2
120	353.1±21.0	346.4±20.0	**342.3±18.4***	**344.8±19.3***	**339.7±16.6***
260	418.8±23.4	413.1±23.2	410.1±21.8	**407.3±23.2***	**401.1±20.6***
372	449.7±24.6/50	445.0±26.1/50	441.6±24.7/50	**435.0±28.1/49***	**425.6±22.0/47***
540	466.4±28.8/50	454.1±33.8/46	457.4±27.6/47	**444.3±35.3/46***	**436.2±25.1/44***
729	431.4±34.3/34	420.3±33.3/38	421.8±38.4/34	409.1±42.8/34	412.9±34.6/33
	♀ (N=65/group unless stated otherwise by a number after /)				
day 0	113.7±5.6	113.6±4.8	113.7±4.5	113.1±4.8	112.8±5.5
36	161.2±7.3	161.6±7.4	160.8±7.5	159.4±7.1	**157.9±7.6***
91	182.5±8.3	182.8±10.0	183.3±8.5	182.4±8.5	**178.0±8.1***
232	211.4±10.6	213.7±11.3	212.2±10.4	210.5±9.7	**205.4±10.1***
372	231.6±14.4/50	234.0±15.0/50	235.3±12.7/50	233.2±14.2/50	228.2±15.6/50
540	275.4±22.8/49	278.2±24.7/49	279.7±23.9/49	273.9±18.9/48	264.2±24.4/47
729	294.4±27.9/44	294.0±26.1/39	305.1±17.9/41	289.4±22.3/38	283.5±23.9/39
data (mean ± SD) obtained from Tables 19-20, pp 126-139; * p <0.05					

Body weights for males at 500 or 1000 mg/kg bw/d were lower than controls and were considered to be treatment related. The differences from controls developed gradually and were first statistically identified at study day 120. At study termination, males at 500 or 1000 mg/kg bw/d weighed 5.2 and 4.3% less than controls, respectively. Body weights of male rats at 50 mg/kg bw/d were slightly lower than controls; however, the difference was not attributed to treatment as they were generally ≥97% of the control weights and the weights were only transiently statistically identified. Rats at 5 mg/kg bw/d also had body weights slightly lower than controls, but were never statistically identified, and were also considered not treatment related. The initial body weights of male rats given either 5 or 50 mg/kg bw/d were slightly lower than rats in the other dose groups.

Body weights for females at 1000 mg/kg bw/d were slightly lower than controls throughout the study and were considered to be treatment-related. As with males, the decrement developed gradually and was maintained at approximately the same level throughout most of the study. At study termination, the high-dose females weighed 3.7% less than controls. Body weights for female rats given 5, 50, or 500 mg/kg bw/d were comparable to controls.

Differences in body weights were reflected in lower body weight gains for males and females at 1000 mg/kg bw/d and also for males given 500 mg/kg bw/d. After one year, body weight gains for males given 500 or 1000 mg/kg bw/d were 4.5 and 7.7% lower than controls, respectively. These decreases remained until study termination, at which time they were 7.7 and 6.3% lower than controls for the 500 and 1000 mg/kg bw/d groups, respectively. Body weight gains for females given 1000 mg/kg bw/d were 2.8% lower than controls after one year and were 3.7% lower at study termination.

C. Food consumption and compound intake

1. Food consumption -

Table 5: Selected food consumption data, g/rat/day

mg/kg bw/d	0	5	50	500	1000
	♂ (N=65/group unless stated otherwise by a number after /)				
days 1-8	16.4±0.9	16.5±0.8	16.6±0.8	16.6±0.7	16.8±0.7/63
71-78	15.4±0.7/63	15.5±0.9/60	14.4±1.2/63	15.8±0.9	**17.0±0.8***
148-155	16.1±0.8/63	16.0±0.7	16.1±0.8	16.3±0.9	**17.0±0.7***
38087	16.4±0.5/64	16.4±0.8	16.1±0.8	16.3±0.9	**17.2±0.6/61***
372-379	16.1±0.7/50	16.1±0.9/50	16.3±0.7/50	**16.6±0.7/49***	**17.5±0.7/44***
484-491	16.8±0.9/50	16.4±1.0/47	16.9±0.8/48	16.8±1.2/47	**18.0±0.9/40***
596-603	16.7±0.7/42	16.8±1.0/43	16.7±1.0/44	17.2±1.1/40	**17.6±0.5/35***
708-715	17.0±1.7/32	17.2±1.4/38	16.9±1.4/32	16.9±1.8/31	**18.2±1.3/33***
data (mean ± SD) obtained from Table 21, pp 140-142; * p ≤0.05					

Starting around week 10, food intake for high-dose males was comparatively higher than control males. The values were typically statistically identified and was considered an effect of treatment.

Food consumption of males at 500 mg/kg bw/d was comparable to controls for the first year of the study. During the second year, feed intake was slightly more than control males sporadically. Feed

consumption for males at 5 and 50 mg/kg bw/d was comparable to control males throughout most of the study period.

Females exposed to XDE-750 had increased food consumption. The higher food intake was not dose-related and was not considered an effect of treatment.

2. Compound consumption (Time-weighed average) - Data are presented in Table 1.

3. Food efficiency -

The results on food efficiency were highly variable, there was no consistent pattern of altered food efficiency related to dose level of XDE-750. Food efficiency decreased over the 13-week time period in both sexes and all dose groups due to slowing of the growth rate while maintaining relatively constant food consumption. Although slightly increased food consumption was noted for males at 1000 mg/kg bw/d and a non-dose related increase for all female dose groups given XDE-750, these minor changes did not result in a consistent pattern of treatment-related altered feed efficiency.

D. Ophthalmoscopic examination - There were no treatment-related findings.

Variable numbers of male and female rats were observed with ocular hemorrhage, engorged blood vessels, pale fundus, cloudy cornea, peri-ocular soiling, cloudy lens, opaque cornea, opaque lens, phthisis bulbi, missing eye and enlarged or protruding eye at the 12- and 24-month intervals. These observations were unrelated to treatment due to their low incidence and/or lack of a dose-response relationship. Peri-ocular soiling was considered to be a non-specific clinical sign while eyes with pale fundus, opaque cornea, opaque lens, cloudy cornea, or cloudy lens were considered to be spontaneous, age-related changes. Phthisis bulbi and missing eye were considered secondary to blood collection via the orbital sinus or due to a spontaneous disease process. At 12 months, 0-6 rats/sex/dose group had peri-ocular soiling and 0-3 rats/sex/dose group were observed with cloudy cornea. Two or less rats/sex/dose group were observed with pale fundus, phthisis bulbi, missing eye, opaque lens or engorged blood vessels. At the end of two years, 4-18 rats/sex/dose were observed with peri-ocular soiling and 9-19 rats/sex/dose were observed with cloudy cornea, with a higher incidence observed in females. These findings were considered unrelated to treatment because of their similar incidence in controls and lack of a dose response. All other observations were noted in ≤4 rats/sex/dose group.

E. Blood analyses - Hematology and clinical chemistry

There were no treatment-related changes in hematologic parameters for male and female rats given XDE-750. The values for each of the parameters evaluated were consistent throughout the first 12 months of the study, with only minor differences from the control values and no consistent changes related to dose level. There was slight inter-animal variability at 18 months and marked inter-animal variability, particularly with regard to WBC count and differential count, at 24 months. However, mean values for any hematologic parameter were not affected in a dose-responsive manner. The individual differences were due to a variety of spontaneous disease processes, primarily leukemia (large granular lymphocyte or mononuclear cell leukemia) and also the stage of leukemic involvement.

Prothrombin time (see Table 6) was equivocally affected for both sexes at 1000 mg/kg bw/d and for males at 500 mg/kg bw/d. However, this was not considered to be toxicologically significant for the following reasons: 1) the effects differed between the sexes; 2) prothrombin times were altered only in the first half of the study; and 3) the changes were small. The minor differences in prothrombin time would be unlikely to be associated with clinical effects and were not associated with other manifestations of clotting disorders.

There were no treatment-related changes in clinical chemistry parameters of male rats at any dose level at any time point.

The AST levels of high-dose females (Table 6) given were slightly increased over the first year of the study and were attributed to ingestion of XDE-750. The AST levels were statistically identified at 3 and 6 months. However, the toxicologic significance of this increase is questionable as: 1) AST levels were not increased at 18 and 24 months; 2) similar elevation was not found in the 13-week study at these dose levels; 3) males were not affected even though they tended to have slightly greater degree for other effects attributed to treatment; and 4) there were no treatment-related histopathologic effects in any organ that could be correlated with the increased AST. As with other parameters, there was minimal inter-animal variability in AST levels through 12 months, but inter-animal variability increased over the remainder of the study. By 24 months, inter-animal variability was moderate and was usually secondary to hepatic involvement by leukemia.

There were no treatment-related changes in electrolytes of male and female rats at any dose level at any time point.

Table 6: Selected hematology and clinical chemistry parameters

mg/kg bw/d	0	5	50	500	1000
	♂ (N=10/group)				
prothrombin time, sec - 3 month	15.2±0.5	15.4±0.8	15.3±0.6	**16.0±0.5***	**16.5±0.7***
6 month	12.4±0.6	12.4±0.4	12.6±0.5	12.8±1.1	12.8±0.4
12 month	11.9±0.6	12.0±0.4	11.9±0.5	12.3±0.6	**12.6±0.4***
18 month	11.5±0.3	11.8±1.1	11.6±0.4	11.7±0.4	11.2±0.6
24 month	11.6±0.5	11.4±0.8	11.5±0.4	11.8±0.7	11.1±0.3
	♀ (N=10/group)				
prothrombin time, sec - 3 month	15.7±0.7	15.5±0.7	15.8±1.6	15.2±1.2	**14.6±0.5***
6 month	12.0±0.8	11.8±0.5	11.9±0.4	11.7±0.3	11.6±0.5
12 month	11.4±0.4	11.4±0.4	11.5±0.4	11.2±0.4	11.0±0.9
18 month	10.9±0.4	10.6±0.5	11.1±1.0	10.8±0.3	10.4±0.7
24 month	10.9±0.3	10.7±0.5	10.9±0.6	10.8±0.6	10.9±0.8
AST, U/L - 3 month	102±10	108±18	107±9	111±19	**133±26***
6 month	112±28	118±27	112±29	118±23	**180±74***
12 month	111±28	104±20	99±18	130±23	**162±77***
18 month	139±51	120±36	100±22	100±18	122±24
24 month	88±15	102±14	129±70	169±113	113±27

* p ≤0.05; data (mean ± SD) obtained from Tables 31, 34, 37, 40, 43, 46, 49, 52, 56, 60, 63, 67, 71, 75, 79; pp 154, 157, 160, 163, 166, 169, 172, 175, 179, 183, 186, 190, 194, 198, 202

F. Urinalysis -

There was a consistent pattern of urinalysis changes for both sexes at 500 or 1000 mg/kg bw/d that was considered treatment-related. The effects included increased urine volume, and decreased specific gravity, pH, protein, and ketones. While these effects are ascribed to treatment, they were somewhat variable in the dose response relationship between these two dose groups and whether or not they were statistically identified. Temporally, the changes developed gradually (being less definitive at 3 months than at 6 months) and were somewhat less definitive by the end of the study, possibly due to the greater variability due to geriatric conditions at this time. These changes were not associated with treatment-related renal histopathology and were considered to be non-adverse.

The urine changes were considered to be adaptive effects to the high levels of XDE-750 in the diet. As noted below (Gross pathology), the ceca of rats at 500 or 1000 mg/kg bw/d were enlarged by semi-solid ingesta, similar to that normally present. As the faeces were normally formed pellets, it is postulated that there was increased colonic water resorption with compensatory renal excretion of the additional water, which led to the somewhat increased urine volume and decreased specific gravity. Decreased urine pH was attributed to renal excretion of XDE-750. As demonstrated in the metabolism study, male rats given 1000 mg [^{14}C] XDE-750/kg bw/d excreted 41% of the administered dose in the urine while those given 50 mg/kg bw/d excreted 50-59% in the urine (Liu, 2003). For both dose groups, the majority was excreted within 24 h as the parent compound. Decreased protein and ketone levels are not considered adverse and were possibly due to dilution of the amounts of these substances normally present in rat urine. Additionally, increased water consumption has been reported in rats given other chemicals that caused enlarged ceca (Leegwater *et al*, 1974; Mann *et al*, 2000). Thus, the changes in volume, specific gravity, protein and ketone levels could be excretion of this addition intake.

Table 7: Selected urine parameters

mg/kg bw/d	0	5	50*	500	1000
	♂ (N=10/group unless stated otherwise by a number after /)				
volume - 3 mon	3.9±1.5	3.4±0.8	3.6±1.0	3.7±1.2	5.0±2.7
6 mon	3.1±1.0	3.3±0.8	3.7±1.5	**5.0±1.0***	**5.7±2.3***
12 mon	4.9±1.2	5.1±4.3	5.7±1.5	**9.0±1.6***	**8.6±2.1***
18 mon	3.8±1.2	3.9±1.1	3.8±0.9	5.6±2.5	6.2±2.6
24 mon	7.3±2.8	6.3±2.9	5.7±2.3	8.5±2.5	**11.3±4.2***
specific - 3 mon	1.081±0.016	1.084±0.007	1.080±0.007	**1.083±0.013**	1.075±0.019
gravity 6 mon	1.082±0.010	1.078±0.008	1.075±0.009	**1.063±0.004***	**1.064±0.015***
12 mon	1.065±0.008	1.066±0.018	1.061±0.009	**1.043±0.008***	**1.049±0.012***
18 mon	1.076±0.010	1.078±0.011	1.070±0.011	**1.058±0.015***	1.065±0.009
24 mon	1.057±0.008	1.060±0.018	1.056±0.011	1.044±0.007	**1.041±0.011***
pH* - 3 mon	2.44000000e+42	6.31000000e+42	2.701000000e+42	1.153000000e+44	1.441000000e+44
6 mon					
12 mon					
18 mon					
24 mon					
	♀ (N=10/group unless stated otherwise by a number after /)				
volume - 3 mon	2.8±0.9	4.2±1.1	3.4±0.7	**4.4±2.1***	**5.5±1.3***
6 mon	3.7±1.5	4.3±2.5	4.4±1.8	5.5±2.4	4.8±1.8
12 mon	5.7±1.8	9.6±10.4	6.8±4.1	8.1±2.8/9	6.6±1.5
18 mon	5.1±0.6	5.8±1.3	5.8±2.3	**9.9±2.9***	**7.3±2.8***
24 mon	8.3±1.9	8.8±2.5	10.0±4.4	12.9±4.8	12.4±5.4
specific - 3 mon	1.072±0.012	1.062±0.014	1.062±0.006	**1.057±0.012***	**1.052±0.013***
gravity 6 mon	1.060±0.022	1.063±0.016	1.057±0.011	1.049±0.010	1.054±0.010
12 mon	1.050±0.014	1.041±0.015	1.048±0.013	1.038±0.007	1.044±0.006
18 mon	1.060±0.005	1.055±0.007	1.057±0.013	**1.041±0.009***	1.053±0.013
24 mon	1.038±0.006	1.041±0.009	1.037±0.007	1.032±0.006	1.033±0.006
pH* - 3 mon	1.13310100e+42	3.42010000e+41	3.320200004e+41	5.310100027e+41	4.600000541e+41
6 mon					
12 mon					
18 mon					
24 mon					

data (mean ± SD) obtained from Tables 31, 34, 37, 40, 43, 46, 49, 52, 56, 60, 63, 67, 71, 75, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99; pp 154, 157, 160, 163, 166, 169, 172, 175, 179, 183, 186, 190, 194, 198, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222
[a] pH values = 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, ≥9.0, respectively
* $p \leq 0.05$

G. Sacrifice and pathology

1. Organ weight -

Table 8: Cecum weight data

mg/kg bw/d			0	5	50	500	1000
			♂ (N=10/group at 12 month; 31-41 at 24 month)				
full	g	12 mon 24 mon	3.851±0.867 5.209±0.787/31	3.373±0.645 4.983±0.963/36	4.116±0.651 5.519±1.047/33	**8.889±1.901*** **9.049±2.311/33***	**14.548±2.738*** **15.501±4.618/33***
	g/100	12 mon 24 mon	0.932±0.194 1.266±0.191/31	0.849±0.183 1.245±0.237/36	1.015±0.150 1.385±0.258/33	**2.220±0.471*** **2.334±0.594/33***	**3.647±0.693*** **3.995±1.177/33***
empty	g	12 mon 24 mon	1.328±0.178 2.400±0.357/31	1.298±0.159 2.375±0.402/36	1.390±0.186 2.340±0.442/33	**2.203±0.416*** **3.123±0.473/33***	**2.545±0.258*** **3.573±0.687/33***
	g/100	12 mon 24 mon	0.321±0.032 0.584±0.090/31	0.326±0.044 0.592±0.090/36	0.343±0.043 0.586±0.103/33	**0.550±0.101*** **0.807±0.132/33***	**0.638±0.065*** **0.925±0.162/33***
			♀ (N=10/group at 12 month; 31-41 at 24 month)				
full	g	12 mon 24 mon	3.790±0.392 3.999±0.607/41	3.636±0.521 4.174±0.576/38	3.570±0.530 4.208±0.657/39	**5.284±1.126*** **5.721±1.115/38***	**11.205±2.615*** **10.438±1.914/39***
	g/100	12 mon 24 mon	1.734±0.109 1.441±0.226/41	1.600±0.231 1.522±0.234/38	1.620±0.203 1.474±0.241/39	**2.408±0.479*** **2.119±0.392/38***	**5.377±1.076*** **3.942±0.768/39***
empty	g	12 mon 24 mon	0.990±0.085 1.862±0.220/41	0.954±0.089 1.889±0.318/38	0.965±0.190 1.957±0.258/39	1.154±0.179 **2.141±0.297/38***	**1.700±0.267*** **2.592±0.315/39***
	g/100	12 mon 24 mon	0.454±0.036 0.671±0.087/41	0.422±0.056 0.687±0.113/38	0.437±0.075 0.685±0.086/39	0.529±0.092 **0.795±0.101/38***	**0.819±0.113*** **0.980±0.145/39***

data (mean ± SD) obtained from Tables 101, 102, 106, 107; pp 224-227, 254-257; * $p \leq 0.05$

Twelve months:
Males and females given 500 or 1000 mg/kg bw/d for 12 months had treatment-related, statistically identified increases in absolute and relative full (including contents) and empty cecal weights. Females were somewhat less affected. Once emptied of ingesta, the cecal weights were also increased, although the degree of increase was less than that for the full cecum.

Twenty-four months:
As at 12 months, the only organ weight considered affected by XDE-750 was the cecum. Males and females given 500 or 1000 mg/kg bw/d for 24 months had treatment-related, statistically identified increases in absolute and relative full (including contents) and empty cecal weights. The difference between the absolute full cecal weights of treated rats and controls was about the same to equivocally less than that found at 12 months. The full cecal weight of males at 1000 mg/kg bw/d remained about 10.3 g greater than controls while that for females was 6.4 g greater vs a 7.4 g difference at 12

months. For rats at 500 mg/kg bw/d, the absolute full cecal weight was 3.8 g greater than controls at 24 months vs 5.0 g greater after 12 months for males, while the female ceca were 1.7 g heavier vs. the 1.5 g heavier at 12 months. However, due to the somewhat greater weight of the ceca of control rats at 24 months, the ratio of treated to control cecal weights was slightly decreased from that found at 12 months. As noted previously, the degree of weight increase of the empty cecum was less than that for the full cecum.

The relative weights of the heart, liver, and ovaries were statistically identified for females at 500 or 1000 mg/kg bw/d. However, these are attributed to the decreased body weight of the females from these dose groups at terminal necropsy. Other weights that were statistically identified were not considered treatment related because they occurred sporadically and lacked dose response relationships.

As expected for Fischer 344 rats after 24 months, there was greater variability in organ weights. This was largely due to neoplasia and particularly affected the weights of the spleen due to frequent occurrence of leukemia. Other organs for which tumor-affected organ weights included the adrenal glands, ovaries, and uterus. While the incidence of neoplasia in these organs was much lower than leukemic involvement of the spleen, the large size of some of the tumors somewhat affected mean organ weight and clearly affected the standard deviation.

2. **Gross pathology** - (see Table 9)

Twelve months:
The only treatment-related effect noted at necropsy was increased size of the cecum of males and females at ≥500 mg/kg bw/d. Several other findings were considered consistent with spontaneous changes commonly found in Fischer 344 rats of this age and husbandry conditions. Most of the observations were of a minor degree or extent of organ involvement and involved only one or two rats in any dose group without any dose-response pattern. The only exception was the lesion of the right optic nerve, which was noted to be of decreased size or missing in about one-half of the rats, but there was no relationship to level of XDE-750 ingested. The optic nerve lesion was attributed to the effects of repeated retro-orbital blood sampling.

The only masses identified at necropsy were found in females and consisted of a pituitary mass, a subcutaneous mass, and five uterine polyps. There was no indication of a dose response and all were considered typical of common tumors of Fischer 344 rats.

Twenty-four months:

As at 12 months, the only treatment-related effect was cecal enlargement, which was observed at 500 and 1000 mg/kg bw/d.

As expected for aged Fischer 344 rats, numerous observations, including many mass-nodules, were made at 24 months. However, none were considered indicative of treatment-related adverse effects and all were consistent with expected geriatric conditions of Fischer 344 rats.

3. **Microscopic pathology** - (see Table 9)

a) Non-neoplastic -

Twelve months:

The only treatment-related histopathologic alteration was a very slight, diffuse hyperplasia of the mucosal epithelium of the cecum of rats at 500 and 1000 mg/kg bw/d. This subtle change was

characterized by increased numbers and crowding of nuclei at the base and sides of the cecal crypts. Neither the thickness of the mucosa, the depth of the crypts, nor thickness of the muscularis were affected in these rats.

All other histopathologic observations found in rats from the 12-month necropsy were considered spontaneous alterations, unassociated with exposure to XDE-750. Most diagnoses were of a minor degree (very slight or slight severity or extent of organ involvement). Most diagnoses involved from 1-3 rats in any dose group, while those that were found at higher incidences did not have a treatment related pattern. With one exception, all were considered to be typical of spontaneous lesions of Fischer 344 rats of this age and husbandry conditions. The exception was the optic nerve lesion, generally unilateral, which was attributed to repeated retro-orbital blood sampling. Varying severities of unilateral optic nerve degeneration, including some in which only little or no nerve tissue was present among connective tissue and vasculature of the retro-bulbar area (termed decreased size, severe) were present, but there was no relationship to XDE-750 ingestion. Many of these rats also had unilateral atrophy of the optic tract in the brain and retinal atrophy in the eye, but these were of generally lesser severity grades than the associated optic nerve lesions.

Twenty-four months:

The only treatment-related histopathologic effect was very slight hyperplasia of the cecal mucosa. However, it was found less frequently than at 12 months and was statistically identified only for high-dose males. There were no tumors that were statistically identified or were considered related to XDE-750 ingestion.

Cecal enlargement of test rodents, particularly rats, has been found following oral administration of many different chemicals or food additives (Bertram, 1996; Cohen & Ito, 2002; Grice & Goldsmith, 2000; Kasahara *et al*, 2002; Leegwater *et al*, 1974; Mann *et al*, 2000; Newberne *et al*, 1988). These materials include modified starches, polyols (sorbitol and mannitol), carrageenan, processed seaweed, wheat and oat bran and several other plant derived materials. Most of these appear to be poorly absorbed materials and colloidal osmotic pressure is considered to underlie the cecal enlargement. Cecal enlargement has been found for many other chemicals, including sodium saccharin, sodium ascorbate, sucralose and lactose that are also presumed to be osmotically active. Cecal enlargement is commonly found with some antibiotics, which are presumed to alter the microflora with subsequent effects on osmotic pressure and/or water transport (Kasahara *et al*, 2002; Cohen and Ito, 2002). While cecal enlargement may be an adaptive phenomenon, there may be functional alterations, such as soft stools, diarrhea, and increased large-bowel mucosal permeability, which are likely to be mediated by the increased osmotic activity of the cecal contents. Some of these chemicals have produced altered serum calcium levels with secondary mineralization in the kidneys (Grice and Goldsmith, 2000; Mann et al, 2000; Newberne *et al*, 1988). Histopathologic lesions of the cecum are often not found but hyperplasia, ulceration, and inflammation have been reported for high dose levels of some chemicals.

While cecal enlargement was the primary effect of XDE-750, rats tolerated up to 1000 mg/kg bw/d quite well. Loose stools or diarrhea were not seen clinically. The cecal enlargement (ie, weight) was essentially similar or slightly less at 24 months than that found at 12 months. There was only very slight hyperplasia of the mucosal epithelium, which was less definitive (ie, lower incidence) after 24 months than at 12 months for high-dose rats. Three of 10 male rats at 500 mg/kg bw/d also had very slight hyperplasia at 12 months but the incidence was similar to controls at 24 months. Serum calcium levels or renal mineralization were not affected by XDE-750. Cecal enlargement in rats appears to be of little significance for man (Cohen and Ito, 2002; Newberne *et al*, 1988; Grice and Goldsmith, 2000). The Joint FAO/WHO Expert Committee on Food Additives noted that "*caecal enlargement without associated histopathological changes is without toxicological significance*" in their review of hydroxypropyl starch (JECFA, 1982). The cecum is fairly large and a functional digestive organ in rats and other herbivores, while it is rudimentary in primates (Bertram, 1996). Doses that produce cecal

enlargement in rodents usually greatly exceed those that humans may ingest. While there was a large number of histopathologic diagnoses made for rats from the 24-month portion of the study, all other diagnoses were considered typical for a geriatric population of Fischer 344 rats. While the incidences varied somewhat, there were no other histopathologic effects - both non-neoplastic and neoplastic-considered related to treatment. There were very few statistically identified histopathologic effects and none were considered related to treatment.

b) Neoplastic - see Table 9

Table 9: Selected gross pathology, histopathology, and tumor data

mg/kg bw/d			0	5	50	500	1000
♂ (N=10/group at 12 month; 50 at 24 month; unless stated /#)							
cecum	increased size	12 mon 24 mon	0	0	0	**924**	**1041**
	mucosal hyperplasia, very slight	12 mon 24 mon	3	2	4	32	**812**
liver	increased size	24 mon	1	2	0	1	**7**
spleen	increased size	24 mon	6	10	12	10	**17**
liver	adenoma carcinoma	24 mon	20	11	1	32	32
thyroid	parafollicular cell adenoma	24 mon	2	38060	38004	38003	6
♀ (N=10/group at 12 month; 50 at 24 month; unless stated /#)							
cecum	increased size	12 mon 24 mon	0	0	0	**69**	**1047**
	mucosal hyperplasia, very slight	12 mon 24 mon	1	2	0	0	**78**
pancreas	focal acinus atrophy, very slight	24 mon	8	37997	37996	38057	16
data obtained from Tables 103-105, 108-110; pp 228-253, 258-323; * p ≤0.05							

Twelve months:

Several tumors were diagnosed for rats from the 12-month interim necropsy but were considered to be unrelated to XDE-750 ingestion. Most tumors were benign and were present in only one rat in any dose group. All were considered typical of common spontaneous tumors of Fischer 344 rats.

Twenty-four months:

Determination of the cause of early removal, ie, spontaneous death or moribund sacrifice, was made on an individual animal basis following histopathologic examination and review of all other changes. The incidence of early removal was unaffected by XDE-750 ingestion. Following histopathologic examination, the underlying causes of early removal were considered similar among all dose groups. The large majority of early removals in all groups, including controls, were due to neoplastic conditions. The two most frequent neoplasms were large granular lymphocyte leukemia (particularly males) and adenomas of the pituitary gland. Both of these types of neoplasms are very common diseases of the Fischer 344 rat and the incidences found in this study were considered unremarkable.

18

Another frequent cause of early removal was large masses of the skin and subcutis, including mammary and other subcutaneous glands. While these were of diverse histotypes, they were also considered typical of common tumors of the Fischer 344 rat and there was no pattern of occurrence in relation to treatment. Low numbers of a variety of other neoplastic and non-neoplastic causes for early removal were also found but these were also considered typical of spontaneous diseases of the Fischer 344 rat and unrelated to treatment.

Most palpable masses were neoplasms, although a low number were non-neoplastic changes. The most common neoplasms in males were derived from the connective tissues of the subcutis (fibromas) but epithelial origin tumors were also present. Most in-life masses in females originated in the mammary gland with the most frequent in all dose groups being fibroadenomas. Lower numbers of malignant neoplasms were found and also neoplasms of organs associated with the skin.

III. DISCUSSION

A. Investigators' conclusions:

"Fischer 344 rats given diets formulated to provide 1000 mg/kg/day of XDE-750, an experimental herbicide, had minor effects attributed to treatment. While there were no adverse effects noted through the in-life portion of the study and survival was not affected, the body weights of rats given 1000 mg/kg/day were slightly decreased and the feed consumption of males was slightly increased. The primary target organ was the cecum, which was enlarged with semisolid ingesta, similar to that normally present. Cecal weights confirmed and quantified the cecal enlargement. Histopathologically, there was very slight hyperplasia of the cecal mucosa. There were changes in several urinalysis parameters that were considered to be not adverse but, rather, adaptive effects to the fluid homeostasis secondary to the cecal enlargement and XDE-750 excretion. The only clinical pathology parameter affected was slightly increased AST levels of females given 1000 mg/kg/day; but this was of uncertain toxicologic significance as it was present only through 12 months. The cecal effects – weights, histopathology – were similar to somewhat less after 24 months ingestion than those found at 12 months. There were no tumors related to XDE-750 ingestion.

Similar effects, but of a lesser degree, were found for rats given 500 mg/kg/day. Again, there were no in-life or survival effects and only males had slightly decreased body weights and equivocally increased feed consumption. Cecal enlargement with increased cecal weight was present for both males and females given 500 mg/kg/day; however, very slight mucosal hyperplasia was noted histopathologically for only 3 of 10 males at 12 months. Secondary adaptive effects on several urinalysis parameters were present for both males and females given 500 mg/kg/day.

No effects attributed to treatment were found for rats of either sex given 5 or 50 mg/kg/day. Thus, 50 mg/kg/day is considered the no-observed-effect level in both sexes (NOEL). The no-observed-adverse-effect level (NOAEL) in females is 500 mg/kg/day; while for males the NOAEL is 50 mg/kg/day due to the slight body weight affects at 500 mg/kg/day. XDE-750 was non-carcinogenic to Fischer 344 rats under the conditions of this study."

B. Reviewer comments:

The study was well conducted and reported. The NOAELs for males and females are 50 and 500 mg/kg bw/d, respectively; based on lower body weight and body-weight gains. The target organ was the cecum. Oral ingestion of XDE-750 resulted in enlarged cecum and increased weight. Histological-ly, the affected ceca showed slight hyperplasia of the mucosa. XDE-750 was non-carcinogenic to Fischer 344 rats under the conditions of this study. This combined chronic toxicity and oncogenicity study in the rat is acceptable and satisfy the guideline requirement for a combined chronic toxicity and oncogenicity study (83-2); OECD 453 in rats.

The neurotoxicity data are presented in a separate report and review.

C. Study deficiencies:

For stability analysis of the test material in the diet, there is no information on the storage conditions.

D. References:

Bertram, TA. 1996. Response of gastrointestinal tract to injury. In *Fundamentals of Toxicologic Pathology* (WH Haschek and CG Rousseaux, Eds), pp 195-251. Academic Press, San Diego.

Cohen, SM and N Ito, 2002. A critical review of the toxicological effects of carrageenan and processed euchema seaweed on the gastrointestinal tract. *Crit Rev Toxicol*, 32, 413-444.

Dryzga, MD and KE Stebbins, 2001. XDE-750: 13-Week dietary toxicity with 4-week recovery study in Fischer 344 rats. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, USA.

Grice, HC and LA Goldsmith, 2000. Sucralose – An overview of the toxicity data. *Food Chem Toxicol*, 38 (Suppl 2), S1-S6.

JECFA (1982). Toxicological evaluation of certain food additives. WHO Food Additives Series 17, Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO *Tech Rep Ser* 683, FAO/WHO, Geneva, p100.

Kasahara, K *et al*, 2002. A chronic toxicity study of josamycin in Fischer 344 rats. *Food Chem Toxicol* 40, 1017-1022.

Leegwater, DC, AP DeGroot, and M van Kalmthout-Kuyper, 1974. The aetiology of caecal enlargement in the rat. *Food Cosmet. Toxicol.* 12, 687-697.

Liu, J, 2003. [^{14}C]XDE-750: Absorption, distribution, metabolism and excretion in male Fischer 344 rats. ABC Laboratories, Inc, Columbia, Missouri. Report of Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, USA.

Mann, SW, MM Yuschak, SJG Amyes, P Aughton, and JP Finn, 2000. A combined chronic toxicity/carcinogenicity study of sucralose in Sprague-Dawley rats. *Food Chem Toxicol*, 38 (Suppl 2), S71-S89.

Newberne, PM, MW Conner, and P Esters, 1988. The influence of food additives and related materials on lower bowel structure and function. *Tox Pathol*, 16, 184-197.

005100
(3)

Aminopyralid (DE750) technical / DOW **~ PROTECTED ~** **Acute neurotoxicity - Rat / 1**
aminopyralid [AMD] / PMRA Sub. No. 2004-0789 **DACO 4.5.12/ OECD IIA 5.7.1**

PMRA Primary Reviewer: Steve Wong, Ph. D. **Signature:**
Fungicide/Herbicide Toxicological Evaluation
Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C)
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Acute neurotoxicity - rat; OPPTS 870.6200 [§81-8]; OECD 424.

PC CODE: 005100 **DP BARCODE**: D305670

TEST MATERIAL (PURITY): XDE-750 (94.5%)

SYNONYMS: aminopyralid; 4-amino-3,6-dichloro-picolinic acid; X660750; XR-750

CITATION: Marable, BR, AK Andrus, and KE Stebbins, December 18, 2001; revised January 28, 2002. XDE-750: acute neurotoxicity study in fischer 344 rats. Laboratory Project ID 011073, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Study conducted May 9 - December 18, 2001. Unpublished

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 46235616), groups of randomly assigned Fischer 344 rats (10/sex) were given a single oral dose of XDE-750 (94.5% purity) in 0.5% aqueous Methocel® at 0, 500, 1000 or 2000 mg/kg bw. The rats were fasted overnight prior to dosing. They were observed daily for signs of toxicity, mortality, and moribundity for 2 weeks after dosing. Body weight was recorded pre-exposure and on days 1, 8, and 15. Clinical examinations were conducted on all animals on test days 2-4. This examination included careful, hand-held evaluations of the skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), swelling, masses and animal behaviour. Daily cage-side examinations were made on each day of the study and to the extent possible the above parameters were evaluated. Functional Observational Battery (FOB) and motor activity testing were performed prior to dosing, and on days 1, 8 and 15. Two week after dosing, the rats were sacrificed and central and peripheral nervous tissues of 5 rats/sex/group were perfusion-fixed *in situ* and subjected to neuropathological examinations.

There were no treatment-related effects on ophthalmoscopy, body weight, the FOB, motor activity, gross pathology, or on neuropathologic evaluation. Clinical observations of rats treated with 2000 mg/kg bw revealed a higher incidence of faecal soiling in males and urine soiling in females compared to controls. However, these effects were transient (most resolving within 3-4 days following treatment) and occurred in the absence of any gross or neuropathologic changes.

From these results, the no-observable-adverse-effect level (NOAEL) for XDE-750 in male and female Fischer 344 rats is 1000 mg/kg bw within the context of an acute neurotoxicity study design. The lowest-observable-adverse-effect level (LOAEL) is 2000 mg/kg bw.

This study is classified acceptable and satisfies the guideline requirement for an acute oral neurotoxicity study (OPPTS 870.6200, DACO 4.5.12) in the rat.

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was conducted in compliance with the US EPA FIFRA GLP and OECD Principles on Good Laboratory Practice.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750 (4-amino-3,6-dichloro-picolinic acid)

Description:	tan powder
Lot/Batch #:	F0031-143, TSN102319
Purity:	94.5 % a.i., determined by a reverse liquid chromatography procedure
CAS #:	no information

2. **Vehicle and/or positive control:**

Vehicle: 0.5% aqueous Methocel®
Positive control: Not performed as part of this study; positive control data were included in the Report (Appendices A-E).

3 **Test animals:**

Species:	rat	
Strain:	Fischer 344	
Age/weight at dosing:	6-wk old; ♂ = 119.2-175.3 g, ♀ = 99.1-130.4 g	
Source:	Charles River Labs Inc, Raleigh, NC, USA	
Housing:	singly in suspended stainless steel cages with mesh floors (2/cage during acclimation)	
Diet:	LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO, USA) *ad libitum*	
Water:	municipal water *ad libitum*	
Environmental conditions:	**Temperature:**	21-23 °C
	Humidity:	40-60 %
	Air changes:	no information
	Photoperiod:	12 h dark/ 12h light (0600-1800 h)
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In life dates** - Start: May 9, 2001 End: December 18, 2001

2. **Animal assignment and treatment** -

Test rats were evaluated for general health and acceptability prior to assigning to groups. The rats were randomly assigned, based on body weight using a computer program, to the test groups noted in Table 1.

TABLE 1: Study design

Dose level (mg/kg bw)	Number of animals							
	Initial		Neurobehavioral studies[b]		Necropsy		Neuropathology	
	♂	♀	♂	♀	♂	♀	♂	♀
0	10	10	10	10	10	10	5	5
500	10	10	10	10	10	10	5	5
1000	10	10	10	10	10	10	5	5
2000	10	10	10	10	10	10	5	5

[a] Control animals received the vehicle at the same volume as the treated animals (10 mL/kg bw)
[b] FOB assessments and motor activity were determined on days 0, 1, 8, and 15.

3. Test substance preparation and analysis

XDE-750 was weighed out in a calibrated beaker, then the vehicle, 0.5% aqueous Methocel®, was added. The resulting suspension was mixed thoroughly for about one minute using a high-speed sonicator. Samples of the dosing preparations were analyzed for stability, homogeneity, and concentration.

Results -

Homogeneity analysis:
Homogeneity analysis of 500 and 2000 mg/kg bw preparations indicated that the top, middle, and bottom mixes were within the target concentrations, 49.8-50.6 mg/mL for the target concentration of 50 mg/mL, and 213-222 mg/mL for the target concentration of 200 mg/mL.

Stability analysis:
The stability of XDE-750 in Methocel® was verified for the 500 and 2000 mg/kg bw dose levels. XDE-750 was confirmed to be stable for 14 and 17 days (90.1 to 106.4% of target concentrations).

Concentration analysis:
A concentration check of the dosing solutions was conducted for all dose levels prior to dosing and was 100%, 109% and 109% of the target for the low, middle and high doses, respectively

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics -

For overall FOB summarization and subjective evaluation, the data were the average ranks for each FOB observation (for males and females at each dose level). For statistical analyses, the incidence of ranked FOB observations, between control and each treated group (for each sex separately), were evaluated by a test of proportions at $\alpha = 0.02$ (Bruning and Kintz, 1977). Due to the high correlation between different ranks (within an observation), if more than one rank within the same observation had significant z scores, the toxicologically most significant rank was reported. Means and standard deviations were calculated by sex for all continuous data and homogeneity of variance was evaluated with Bartlett's test at $\alpha = 0.01$ (Winer, 1971). There were no significant departures from homogeneity of variance at this α level. The study design had two sexes and four major data collection periods:

baseline, days 1, 8 and 15. Initial statistical analyses were factorial repeated-measure analyses to account for data from both sexes at all time periods in one analysis. By using sex as a factor, the statistical power of the test was increased. The inclusion of baseline data in the analysis makes relevant only the analyses which include factors of both treatment and time (Bonate, 2000). The following interactions were examined:

Treatment × Time - A significant p value indicates that, taken together, both males and females were affected by treatment at some time point.

Treatment × Time × Sex - A significant p value indicates that treatment effects were different between males and females at some time point.

Treatment × Time × Epoch (motor activity only) - A significant p value indicates that the within-session distribution of motor activity counts was affected by treatment at some time.

The type I error rate (α) per comparison was set at 0.05 for all continuous data. Subsequent analyses (linear contrasts) were conducted at $\alpha = 0.02$ following a significant primary analysis. The FOB z-test of proportions was conducted at $\alpha = 0.02$ to reduce the rate of false declarations considering the large number of pair-wise comparisons, ie more than 384 comparisons (at least 3 comparisons/sex × 2 sexes × 4 time-points × 16 ranked observations). This overall approach is consistent with the recommendations proposed by Tukey *et al* (1985), Mantel (1980) and USEPA (1991). Probability values are reported without correction.

C. METHODS:

1. Clinical observations:

At dosing, over-night fasted rats were given a single oral dose by gavage. The administration volume was 10 mL/kg bw. After dosing, the animals were observed daily for clinical signs of toxicity, mortality, and moribundity. On days 2, 3, and 4, hand-held evaluations were carried out on the skin, mucous membranes, respiration, nervous system function, swelling, masses, and behaviour.

2. Neurobehavioral studies:

The neurobehavioural evaluation consisted of a functional observation battery (FOB) and determination of motor and locomotor activity. The evaluation was carried out in all rats on days 0, 1, 8, and 15. The FOB, usually performed at the same time each day, included hand-held and open-field observations and measurements of grip performance, landing foot splay, and rectal temperature.

a. Motor activity evaluation:

Motor activity evaluation was performed using 24 motor activity cages, visually isolated from each other, located in a quiet, dimly-lit room. Each motor activity cage consisted of a clear plastic circular alley. An infrared photobeam bisected the cage so that the beam crossed the alley in two locations. All test sessions consisted of six 8-minute epochs, totalling 48 minutes of testing per rat. This duration was chosen based on the results of validation studies performed in the testing laboratory. Total activity counts for each epoch were recorded. Each beam break that lasted more than 100 msec and followed an interval between beam breaks that was greater than 100 msec, constituted an activity count. These minimum durations were set to discount activities such as tail-flicking, rearing, head-bobbing, etc. Motor activity was monitored by a computerized system located in an adjoining room. Motor activity counts were reported as their square roots to minimize problems of heterogeneity of variance and departure from normality that commonly occured from treatment (Pryor *et al*, 1983). Positive control

data for the motor activity test and a detailed description of the apparatus were presented in the Report.

b. FOB evaluations: The following FOB parameters were evaluated:

Cage-side observations	**Hand-held observations**	**Open field observations**
abnormal body movement (tremors, convulsions)	palpebral closure	level of activity (ambulatory and rearing)
abnormal behaviour (circling, stereotypy)	lacrimation	response to sharp noise
posture	pupil size	response to touch
resistance to removal form cage	pupil reactivity	response to tail pinch
Measurements	salivation	urination
hindlimb grip strength	muscle tone	defecation
forelimb grip strength	extensor thrust response	gait evaluation
landing foot-splay	reactivity to handling	
rectal temperature		
Categorical observation (anytime during the FOB)		
skin/fur/mucous membrane	behaviour	respiration
muscle	eye	faeces
soiling	posture	general abnormalities

3. **Body weight:**

Body weight of individual animals were recorded prior to dosing, and on days 1, 8, and 15.

4. **Food consumption:** Food intake of the animals was not recorded.

5. **Ophthalmoscopy:**

Prior to dosing, all rats were examined by a veterinarian using indirect ophthalmoscopy. One drop of 0.5% tropicamide ophthalmic solution was instilled in each eye to produce mydriasis prior to the indirect ophthalmic examination. At necropsy, the eyes were examined using a moistened glass slide.

6. **Sacrifice and pathology:**

At study termination, five randomly selected rats per sex per group were sacrificed (under CO_2 anaesthesia) and perfusion fixed with 0.05 M phosphate buffer containing sodium nitrite followed by a phosphate buffered solution of 1.5% glutaraldehyde - 4% formaldehyde (c. 540 mOs). After sacrifice, all visible organs were assessed for gross pathological changes. The brain, head, spinal column with spinal cord, fore- and hind-limbs, and tail were trimmed to remove excessive skin and muscle, and muscles from the hind-limbs were reflected to further expose the nerves. All tissues were immersed in the glutaraldehyde/formaldehyde fixative. In addition, thoracic and abdominal viscera were collected and preserved in the glutaraldehyde/formaldehyde fixative. Tissues/organs (see below) from the control and high-dose animals were collected for histologic/neuropathologic examination. The remaining rats not selected for perfusion fixation were sacrificed under CO_2 anaesthesia and a standard set of tissues were saved in neutral phosphate-buffered 10% formalin.

Tissues for neuropathologic evaluation were prepared from all rats in the control and high-dose groups. Nine cross-sections of the brain were prepared from the olfactory bulb, cerebrum (frontal, parietal, temporal and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, and

medulla oblongata. In addition, sections were prepared from the trigeminal ganglion and nerve, pituitary gland, eyes with optic nerves, spinal cord (cervical and lumbar), olfactory epithelium, and skeletal muscles (gastro-cnemius and anterior tibial). These tissues were processed by standard histologic procedures, embedded in paraffin, sectioned ~6 µm thick and stained with hematoxylin and eosin. Spinal nerve roots (cervical and lumbar), dorsal root ganglia (cervical and lumbar), and peripheral nerves (sciatic, tibial (proximal and distal - at the knee and calf muscle branches) and sural) were osmicated, embedded in epoxy resin, sectioned approximately 2-3 µm thick and stained with toluidine blue.

II. RESULTS

A. Observations :

1. Clinical signs of toxicity -

Clinical observations revealed an increased incidence of faecal soiling in high-dose males and urine soiling in high-dose females. Of the eight cases of faecal soiling in high-dose males, seven resolved by days 3-4, and the remaining case had resolved by day 8. Four high-dose females had urine soiling on test day 2, all of which resolved by days 3-4. Although these soiling effects were correlated with high-dose treatment, the relevance with regard to neurotoxicity is unclear. Because the soiling seen in high-dose animals was transient, most resolved within 3-4 days of treatment, and occurred in the absence of gross/neuropathological changes, it seemed probable that these effects were short-lived and as such would not be expected to result in permanent changes in function or health.

2. Mortality - There were no deaths.

B. Body weight and weight gain: There were no treatment-related effects on body weight.

C. Food consumption: Not measured.

D. FOB and motor activity evaluations:

There were no toxicologically significant FOB observations related to treatment. The distribution of motor activity counts within each session was not significantly affected by treatment.

F. Sacrifice and pathology

1. Brain weight - No brain weight data were presented.

2. Gross pathology - There were no treatment-related gross pathological findings.

3. Microscopic pathology -

There were no treatment-related neuropathology and other histopathological findings (based on the examination of the nervous tissues of 5 animals/sex/group).

III. DISCUSSION

A. Investigators' conclusions:

"Treatment with XDE-750 did not affect cage-side, FOB hand-held and open-field observations, body

weight, hindlimb grip performance, forelimb grip performance, rectal temperature or landing foot splay, either in males or in females, at any dose. A transient soiling effect was seen in high-dose animals that resolved within eight days of treatment.

XDE-750 did not affect motor activity either in males or in females at any dose or at any time during the study.

The results of the neuropathologic evaluation indicated that XDE-750 had no effect on the central or peripheral nervous system. The neuropathological NOEL for the study was greater than 2000 mg/kg for both males and females.

From these results, we conclude that the no-observable-effect level (NOEL) for XDE-750 in male and female Fischer 344 rats is 1000 mg/kg within the context of an acute neurotoxicity study design. We additionally recognize that acute soiling effects notwithstanding, no effects indicative of neurotoxicity were seen at the highest dose used, 2000 mg/kg."

B. Reviewer comments:

Documentations demonstrating the proficiency of the investigators on the assessment of FOB and neurotoxicity were included in the Report. Also, reports on validation of motor activity using positive control compounds were included.

Brain weights were not determined in the study. Histopathological examination of the nervous tissues did not reveal any treatment-related findings. The absence of the brain-weight data therefore would not impact on the overall conclusions of the study. The study authors' conclusions are acceptable.

C. Study deficiencies:

Dosing preparations were stored for 14 and 17 days prior to stability analyses. The storage conditions were not given. The lack of this information and the absence of brain weight data should not impact on the overall conclusions of the study. This study is classified acceptable and satisfies the guideline requirement for an acute oral neurotoxicity study (DACO 4.5.12) in the rat.

IV. REFERENCES

Bonate, PL, 2000. *Analysis of pretest-post-test designs*. Chapman & Hall/CRC, Boca Raton, FL.

Bruning, JL and BL Kintz, 1977. *Computational Handbook of Statistics*. Scott, Foresman and Co, IL

Mantel, N. 1980. Assessing laboratory evidence for neoplastic activity. *Biometrics*, 36, 381-399.

Pryor, GT, ET Uyeno, HA Tilson, and CL Mitchell, 1983. Assessment of chemicals using a battery of neurobehavioral tests: A comparative study. *Neurobehav Toxicol Teratol*, 5, 91-117.

Tukey, JW, JL Ciminera, and JF Heyse, 1985. Testing statistical certainty of a response to increasing doses of a drug. *Biometrics*, 41, 295-301.

US EPA, 1991. Environmental Protection Agency. Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Addendum 10, Neurotoxicity Series 81, 82, and 83, PB91-154617.

Winer, BJ, 1971. *Statistical principles in experimental design*. McGraw-Hill Co., New York, NY.

005100
(4)

PMRA Primary Reviewer: Steve Wong, Ph. D. **Signature:**
Fungicide/Herbicide Toxicological Evaluation
Section, Health Evaluation Division **Date** Aug 31 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C)
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: 1-Year neurotoxicity - Rat, OPPTS 870.6200 [§81-8]; OECD 424.

PC CODE: 005100 **DP BARCODE**: D305670

TEST MATERIAL (PURITY): XDE-750 (94.5%)

SYNONYMS: aminopyralid; 4-amino-3,6-dichloro-picolinic acid; X660750; XR-750

CITATION: Maurrisen, JP, AK Andrus, KA Johnson, and D Dryzga, September 22, 2003. XDE-750: chronic neurotoxicity study in Fischer 344 rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Unpublished

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

A one-year neurotoxicity study (MRID 46235617) was conducted as part of a two-year chronic toxicity/oncogenicity study to assess the effects of dietary exposure to XDE-750 at levels of 0, 5, 50, 500, and 1000 mg/kg bw/d in male and female Fischer 344 rats. The neurotoxicity subgroup contained 10 rats/sex/dose and was evaluated pre-exposure, and at 1, 3, 6, 9, and 12 months of exposure using a functional observational battery (FOB), determinations of grip performance, rectal temperature, landing foot splay, and an automated test of motor activity. Following 12 months of exposure, five rats/sex from the control and high-dose groups were perfused, and tissues from the central and peripheral nervous system were submitted for neuropathologic examination.

No treatment-related effects were seen on any of the FOB parameter assessed, or on motor activity at any time during the study. Within the neurotoxicity subgroup, no significant treatment-related effects were seen on body weight, though significant body weight effects were seen in males treated with 1000 or 500 mg/kg bw/d (5.3 and 3.2% less than control; respectively) when all study animals (n = 65/sex/group; chronic neurotoxicity/chronic toxicity/oncogenicity) were considered. An increase in the level of defecation during the open-field activity of the FOB was seen in males of the high-dose group, and less consistently in the mid-dose groups. This effect was not considered a neurotoxic effect, but rather reflected a non-specific effect.

There were no treatment-related gross or histopathologic findings in the central or peripheral nervous system following one year of dietary exposure to XDE-750. In summary, there were no effects of XDE-750 on any parameter that would suggest a neurotoxic effect. Therefore, the chronic dietary NOAEL for XDE-750 neurotoxicity in Fischer 344 rats was 1000 mg/kg bw/d, the highest dose level tested.

This study is classified acceptable and satisfies the guideline requirement for a one-year oral neurotoxicity study (DACO 4.5.13) in the rat.

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was conducted in compliance with the US EPA FIFRA GLP and OECD Principles on Good Laboratory Practice.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material**:

Description:	tan powder
Lot/Batch #:	F0031-143, TSN102319
Purity:	94.5 % ai, determined by a reverse liquid chromatography procedure
CAS #:	150114-71-9

2. **Vehicle and/or positive control**:

LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO, USA)

3 **Test animals**:

Species:	rat	
Strain:	Fischer 344	
Age/weight at dosing:	6-wk old: ♂ = 119.2-175.3) g, ♀ = 99.1-130.4) g	
Source:	Charles River Labs Inc, Raleigh, NC, USA	
Housing	2 per suspended stainless steel cages with mesh floors	
Diet:	LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO, USA) *ad libitum*	
Water:	municipal water *ad libitum*	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	40-70 %
	Air changes:	12-15 exchanges/h
	Photoperiod:	12 h dark/ 12h light (0600-1800 h)
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In life dates** - Start: July 30, 2001 End: August 21, 2002

2. **Animal assignment and treatment** -

Table 1: Study design

Dose level, mg/kg bw/d (actual)	Number of animals							
	Initial		Neurobehavioral studies[a]		Necropsy		Neuropathology	
	♂	♀	♂	♀	♂	♀	♂	♀
0 (0)	10	10	10	10	10	10	5	5
5 (♂ = 5.11, ♀ = 5.02)	10	10	10	10	10	10	5	5
50 (♂ = 51.1, ♀ = 50.2)	10	10	10	10	10	10	5	5

500 (♂ = 511.5, ♀ = 503.4)	10	10	10	10	10	10	5	5
1000 (♂ = 1025.4, ♀ = 1006.5)	10	10	10	10	10	10	5	5
[a] FOB assessments and motor activity were determined on days 0, 1, 8, and 15.								

Test rats were evaluated for general health and acceptability prior to assigning to groups. The rats were randomly assigned, based on body weight using a computer program, to test groups noted in Table 1. The dose levels chosen were based on preliminary results of a 13-week dietary toxicity with 4-week recovery study in rats. Male rats given 1000 mg/kg bw/d for 13 weeks had epithelial hyperplasia of the cecum and ileum. In addition, cecal weights of males and females given 1000 mg/kg bw/d for 13-weeks were significantly increased. Therefore, a high-dose of 1000 mg/kg bw/d was chosen for this study. The other doses were expected to provide dose response data for any treatment-related effects observed in the high-dose group.

3. **Test substance preparation and analysis**

Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were mixed periodically throughout the study based on stability data. Initial concentrations of test material in the diet were calculated from pre-exposure body weights and feed consumption data. Subsequently, the concentrations of XDE-750 in the feed were adjusted weekly for the first 13 weeks of the study and at 4-week intervals thereafter, based upon the most recent body weight and feed consumption data. Stability of the test compound in the diet was determined, and concentration and homogeneity were periodically verified.

Results -

Homogeneity analysis:
Homogeneity tests were conducted with the test diet preparations at 5 and 1000 mg/kg bw/d on 9 occasions as well as with the 7% pre-mix and the 50 mg/kg bw/d preparation on one occasion. The analytical values were usually within 10% of the targets, with the exception of one 5 mg/kg bw/d preparation.

Stability analysis:
A stability test was conducted with the control and 7% pre-mix after 87 days. All control diet showed below the limit of quantitation of the test material. The 7% pre-mixes showed stability values of 90, 102, and 96.5% of the initial concentration after 41, 54, and 84 days of storage, respectively.

Concentration analysis:
Concentration analyses were carried out with test diets prepared on 9-10 occasions. The analytical concentrations varied from 90 to 117 of the target concentrations, with the exceptions of 2 preparations which were 86.4 and 86.9 % of the target concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics** -

For overall FOB summarization and subjective evaluation, the data were the average ranks for each FOB observation (for males and females at each dose level). For example, there were five levels of muscle tone ranging from "none" to "exaggerated" and these levels were assigned points from 1 to 5. The score for each level was equal to the level point multiplied by the incidence for that level. The average score for a specific FOB observation was equal to the sum of scores for each level/number of

rats. The average scores were descriptive only, and were not quantitatively analyzed.

For statistical analyses, the incidence of ranked FOB observations, between control and each treated group (for each sex separately), were evaluated by a z-test of proportions at $\alpha = 0.02$ (Bruning and Kintz, 1987). Due to the high correlation between different ranks (within an observation), if more than one rank within the same observation had significant z scores, the toxicologically most significant rank was reported.

Means and standard deviations were calculated by sex for all continuous data and homogeneity of variance was evaluated with Bartlett's test at $\alpha = 0.01$ (Winer, 1971). There were no significant departures from homogeneity of variance at this α level. The study design had two sexes and six major data collection periods: baseline, months 1, 3, 6, 9, and 12. Initial statistical analyses were factorial repeated-measure analyses to account for data from both sexes at all time periods in one analysis. By using sex as a factor, the statistical power of the test was increased. The inclusion of baseline data in the analysis made relevant only the analyses which included factors of both treatment and time (Bonate, 2000). The following interactions were examined:

Treatment × Time - A significant p value indicated that, taken together, both males and females were affected by treatment at some time point.

Treatment × Time × Sex - A significant p value indicated that treatment effects were different between males and females at some time point.

Treatment × Time × Epoch (motor activity only) - A significant p value indicated that the within-session distribution of motor activity counts was affected by treatment at some time.

The type I error rate (α) per comparison was set at 0.05 for all continuous data. Subsequent analyses (linear contrasts) were conducted at $\alpha = 0.02$ following a significant primary analysis. The FOB z-test of proportions was conducted at $\alpha = 0.02$ to reduce the rate of false declarations considering the large number of pair-wise comparisons, ie more than 384 comparisons (at least 3 comparisons/sex × 2 sexes × 4 time-points × 16 ranked observations). The overall approach was consistent with the recommendations proposed by Tukey *et al*, 1985, Mantel, 1980 and US EPA, 1991. Probability values were reported without correction.

C. METHODS:

1. Mortality and clinical observations:

Animals were observed twice daily for the availability of feed and water, and mortality and moribundity. Animals were observed once daily with a detailed physical examination designed to detect signs of toxicity without removing animals from the home cage.

2. Neurobehavioral studies:

The neurobehavioral evaluation consisted of a functional observation battery (FOB) and determination of motor and locomotor activity. The evaluation was carried out in all rats on days 0, 1, 8, and 15. The FOB, usually performed at the same time each day, included hand-held and open-field observations and measurements of grip performance, landing foot splay, and rectal temperature.

a. Motor Activity Evaluation:

Motor activity evaluation was performed using 24 motor activity cages, visually isolated from each other, located in a quiet, dimly-lit room. Each motor activity cage consisted of a clear plastic circular

alley. An infrared photo-beam bisected the cage so that the beam crossed the alley in two locations. All test sessions consisted of six 8-minute epochs, totaling 48 minutes of testing per rat. This duration was chosen based on the results of validation studies performed in the testing laboratory. Total activity counts for each epoch were recorded. Each beam break that lasted more than 100 msec and followed an interval between beam breaks that was greater than 100 msec, constituted an activity count. These minimum durations were set to discount activities such as tail-flicking, rearing, head-bobbing, etc. Motor activity was monitored by a computerized system located in an adjoining room. Motor activity counts are reported as their square roots to minimize problems of heterogeneity of variance and departure from normality that commonly occur from treatment (Pryor *et al*, 1983). Positive control data for the motor activity test and a detailed description of the apparatus were presented in the Report.

b. FOB Evaluations: The following FOB parameters were evaluated:

Cage-side observations	**Hand-held observations**	**Open field observations**
abnormal body movement (tremors, convulsions)	lacrimation/ chromodacryorrhea	level of activity (ambulatory and rearing)
abnormal behavior (circling, stereotypy)	palpebral closure	response to sharp noise
posture	pupil size	response to touch
resistance to removal form cage	pupil reactivity	response to tail pinch
biting	piloerection	pupil response
feces consistency	fur appearance	urination
Measurements	salivation	defecation
rectal temperature	extensor thrust response	gait abnormalities/posture / score
hind-limb grip strength	reactivity to handling	grooming
forelimb grip strength		convulsions / tremor
landing foot-splay		bizarre/stereotypic behavior
hind-limb extensor strength		backing time to first step
Categorical observation (anytime during the FOB)		
skin/fur/mucous membrane color	behavior	respiration rate
muscle tone	eye prominence	feces
soiling (red/crusty deposits)	posture	general abnormalities

3. **Body weight**:

Animals were weighed weekly for the first 13 weeks and then monthly thereafter. For this report, only body weights taken prior to the FOB were examined.

4. **Food consumption:**

Feed consumption was measured weekly for the first 13 weeks and then monthly thereafter. Test material intake (TMI) was calculated using test material concentrations in the feed, actual body weights and feed consumption in the following equation:

$$TMI = \frac{\text{(food consumption, g/d) x (1000 mg/g) x (\% of XDE-750 in feed/100)}}{\text{[(current bw, g + previous bw, g)/2]/(1000 g/kg)}}$$

37

5. Ophthalmoscopy:

All eyes were examined by a veterinarian prior to dosing and at the end of the study period.

6. Sacrifice and pathology:

At study termination, five randomly selected, over-night fasted rats per sex per group were sacrificed (under CO_2 anaesthesia) and perfusion fixed with 0.05 M phosphate buffer containing sodium nitrite followed by a phosphate buffered solution of 1.5% glutaraldehyde - 4% formaldehyde (c. 540 mOs). After sacrifice, all visible organs were assessed for gross pathological changes. The brain, head, spinal column with spinal cord, fore- and hind-limbs, and tail were trimmed to remove excessive skin and muscle, and muscles from the hind-limbs were reflected to further expose the nerves. All tissues were immersed in the glutaraldehyde/formaldehyde fixative. In addition, thoracic and abdominal viscera were collected and preserved in the glutaraldehyde/formaldehyde fixative. Tissues/organs (see below) from the control and high-dose animals were collected for histologic/neuropathologic examination. The remaining rats not selected for perfusion fixation were sacrificed under CO_2 anaesthesia and a standard set of tissues were saved in neutral phosphate-buffered 10% formalin.

Tissues for neuropathologic evaluation were prepared from all rats in the control and high-dose groups. Nine cross-sections of the brain were prepared from the olfactory bulb, cerebrum (frontal, parietal, temporal and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, and medulla oblongata. In addition, sections were prepared from the trigeminal ganglion and nerve, pituitary gland, eyes with optic nerves, spinal cord (cervical and lumbar), olfactory epithelium, and skeletal muscles (gastro-cnemius and anterior tibial). These tissues were processed by standard histologic procedures, embedded in paraffin, sectioned ~6 µm thick and stained with hematoxylin and eosin. Spinal nerve roots (cervical and lumbar), dorsal root ganglia (cervical and lumbar), and peripheral nerves (sciatic, tibial (proximal and distal - at the knee and calf muscle branches) and sural) were osmicated, embedded in epoxy resin, sectioned approximately 2-3 µm thick and stained with toluidine blue.

II. RESULTS

A. Mortality and clinical signs of toxicity :

There were no deaths. No treatment related clinical signs were seen throughout the study.

B. Body weight and weight gain:

There were no treatment-related effects on body weight on the sub-set of animals (10/sex/group) assigned to neurotoxicity assessment. However, when all the animals of the long-term toxicity study (65/sex/group) were considered, the body weights of males at 500 and 1000 mg/kg bw/d were comparatively lower than the control males. The finding was reported in the 2-year rat chronic toxicity/ oncogenicity study review.

C. Food and test compound consumption:

Food consumption and test compound consumption data for all animals were reported in the 2-year rat chronic toxicity/ oncogenicity study review. Starting around week 10, food consumption for high-dose males was comparatively higher than control males. The values were typically statistically identified and was considered an effect of treatment.

D. FOB and motor activity evaluations:

An increase in the level of defecation in male rats during the open-field observation appeared to be treatment-related. This was apparent in the high-dose males beginning at month 3, and remained consistent through month 12. This effect also appeared to be dose responsive in males across the two middle dose groups beginning at month 6. Defecation also appeared to be increased in the 500 mg/kg bw/d group at months 6 and 9. This effect was probably related to the increased size of the cecum as seen in the chronic/oncogenicity portion of this study. Additionally, a decreased level of open field activity was statistically identified in the high-dose males at month 12. This finding could not be reliably attributed to treatment, as it was exclusive to one dose group, at one FOB time point, and in one sex. Also, a decrease in the level of activity in high-dose males (or any other dose group) is not supported by the motor activity data collected at any time point. The distribution of motor activity counts within each session was not significantly affected by treatment.

There were no toxicologically significant FOB observations related to treatment.

Table 2: Defecation and selected level of activity data for males

	mg/kg bw/d (10/group) - ♂																								
	0					5					50					500					1000				
defecation grade	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
pre-exposure	10	0	0	0	0	10	0	0	0	0	8	2	0	0	0	9	1	0	0	0	9	1	0	0	0
month 1	8	2	0	0	0	7	3	0	0	0	7	3	0	0	0	7	3	0	0	0	7	3	0	0	0
month 3	4	5	1	0	0	3	7	0	0	0	5	5	0	0	0	4	4	1	1	0	3	1	4	2	0
	1.7[a]					1.7					1.5					1.9					**2.5**				
month 6	4	5	1	0	0	5	5	0	0	0	1	6	3	0	0	3	3	3	1	0	1	4	2	2	1
	1.7					1.5					**2.2**					**2.2**					**2.8**				
month 9	6	4	0	0	0	6	4	0	0	0	3	7	0	0	0	4	2	2	2	0	2	5	2	1	0
	1.4					1.4					1.7					**2.2**					**2.2**				
month 12	4	6	0	0	0	6	4	0	0	0	2	7	1	0	0	3	4	3	0	0	0	5	5	0	0
	1.6					1.4					1.9					2					**2.5**				
activity grade	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
month 12	4	6	0	0	0	6	4	0	0	0	2	7	1	0	0	3	4	3	0	0	0	5	5	0	0
	1.7					1.5					1.4					1.5					**1.1**				

defecation grades: 0 = none; 1 = minimal; 2 = moderate; 3 = pronounced; 4 = exaggerated
values represent the number of animals showing specific grade; [a] = average ranks of observations, in **bold** if the average ranked value differs from the control value by 0.5
Data were extracted from Tables 7. & 14-19.

F. Sacrifice and pathology

1. Brain weight - No brain weight data were presented.

2. Gross pathology -

At necropsy, the cecum of rats at 500 or 1000 mg/kg bw/d was enlarged due to increased amount of normal-appearing contents. The cecal enlargement was quantified in the rats (10/sex/dose level) from the chronic toxicity portion of this study (see review of the chronic/oncogenicity study). There were no other observations made at necropsy that were attributed to the ingestion of XDE-750.

3. Microscopic pathology -

There were no treatment-related neuropathology and other histopathological findings (based on the examination of the central and peripheral nervous tissues of 5 rats/sex/group).

III. DISCUSSION

A. Investigators' conclusions:

"Twelve months of dietary exposure up to 1000 mg/kg/day XDE-750 did not affect body weight in either the male or female Fischer 344 rats assigned to the neurotoxicity subgroup (n = 10/sex/dose). However, significant decreases in body-weights, in males treated with 1000 or 500 mg/kg/day (5.3 and 3.2% less than control; respectively), were seen when all study animals were considered after one year of treatment (n = 65/sex/group; Johnson et al., 2004).

For the FOB, in both scored and non-scored observations, only an increase in the level of defecation during open-field activity correlated with treatment, and this effect reliably occurred only in the high-dose males. Treatment did not affect grip performance, rectal temperature, landing foot splay, motor activity, or ophthalmic observations in either male or female rats, at any point during the study.

There were no treatment-related gross or histopathologic observations in the central or peripheral nervous systems of rats administered XDE-750, and the NOEL for neuropathology was at least 1000 mg/kg/day for both male and female Fischer 344 rats.

Given the absence of neuropathologic findings in the central and peripheral nervous system, and the lack of effect on all other parameters suggestive of neurotoxicity, the increase in defecation seen in high-dose males is not considered to be the result or expression of neurotoxicity. Therefore, the NOEL for neurotoxicity in Fischer 344 rats following a one year dietary exposure to XDE-750 is greater than 1000 mg/kg/day."

B. Reviewer comments:

The study was properly conducted and reported. The study authors' conclusions are acceptable. Documentations demonstrating the proficiency of the investigators on the assessment of FOB and neurotoxicity were included in the report. Also, reports on validation of motor activity using positive control compounds were included. This study is classified acceptable and satisfies the guideline requirement for a one-year oral neurotoxicity study (DACO 4.5.13) in the rat.

C. Study deficiencies: There are no deficiencies.

IV. REFERENCES

Bonate, PL, 2000. *Analysis of pretest-post-test designs*. Chapman & Hall/CRC, Boca Raton, FL.

Bruning, JL and BL Kintz, 1987. *Computational Handbook of Statistics*. Scott, Foresman and Co, IL

Mantel, N, 1980. Assessing laboratory evidence for neoplastic activity. *Biometrics*, 36, 381-399.

Pryor, GT, ET Uyeno, HA Tilson, and CL Mitchell, 1983. Assessment of chemicals using a battery of neurobehavioral tests: A comparative study. *Neurobehav Toxicol Teratol*, 5, 91-117.

Tukey, JW, JL Ciminera, and JF Heyse, 1985. Testing statistical certainty of a response to increasing doses of a drug. *Biometrics*, 41, 295-301.

US EPA, 1991. Environmental Protection Agency. Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Addendum 10, Neurotoxicity Series 81, 82, and 83, PB91-154617.

Winer, BJ, 1971. *Statistical principles in experimental design*. McGraw-Hill Co., New York, NY.

00-1166
(5)

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** Steve Wray for
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C)
Date 7/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Study in Mice; OPPTS 870.3100 (rodent); OECD 408.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Stebbins K.E., et al (2001) XDE-750: 13-Week Dietary Toxicity Study in CD-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 001240, December 20, 2001. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a subchronic toxicity study (13 weeks) (MRID 46235618), technical XDE-750, purity 94.5%, was administered to 10 CD-1 mice per sex per group in the diet at dose levels of 0, 10, 100, 500 or 1000 mg/kg bw/day (equal to 0, 10.2, 101, 512 or 1020 mg/kg bw/day for males, and 0, 102, 103, 515 or 1020 mg/kg bw/day for females). There were no treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, organ weights, hematology, clinical chemistry, gross pathology or histopathology.

The LOAEL could not be determined since there were no treatment-related findings observed for any of the parameters evaluated in this study. The NOAEL is 1000 mg/kg bw/day (1020.0/1020.0 mg/kg bw/day).

This subchronic toxicity study in the mouse is acceptable, and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in the mouse.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-143; TSN102319
Purity:	95.4% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5002 Certified Rodent Lab Diet).

3 **Test Animals:**

Species:	Mouse	
Strain:	CD-1	
Age/weight at study initiation:	6-7 weeks of age; Males, 25.9 g to 33.2 g; Females, 21.8 g to 27.3 g.	
Source:	Charles River Laboratories, Inc., Portage, MI.	
Housing:	Individually housed in suspended stainless steel cages with wire-mesh floors.	
Diet:	Purina Certified Rodent Lab Diet #5002 in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	21.7-22.5°C
	Humidity:	48.2-52.2%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	One week.	

B. STUDY DESIGN:

1. **In Life Dates:** January 19, 2001 to April 20, 2001.

2. **Animal Assignment:** Animals were stratified by body weight and then randomly assigned to the test groups noted in Table 1 using a computer program .

TABLE 1 - Study Design

Test Group	Conc. in Diet (mg/kg bw/day)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	37986	10	10
Low	10	10.2/10.2	10	10
Mid-low	100	101/103	10	10
Mid-high	500	37988	10	10
High	1000	1020/4840	10	10

The high dose level of 1000 mg/kg bw/day represented the limit dose. The remaining dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group and to ensure definition of a no-observed-effect level (NOEL).

3. **Diet Preparation and Analysis:** Fresh diets were prepared weekly based on the most recent body weight and food consumption data. Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Stability of the test material in diet for up to 21 days was demonstrated in the 4-week dietary toxicity study in mice (Study No. 001048), and so stability analyses were not conducted in the 13-week mouse study. Homogeneity of mixing was determined for the low-dose female diets and high-dose male diets prior to study initiation, approximately mid-way through the study and near study termination. Actual test material concentration in the diet was determined for all dose levels from test diets prepared just prior to study initiation, approximately mid-way through the study and near the end of the study.

Results - Homogeneity Analysis: The homogeneity of the test material was evaluated in the low-dose female and the high-dose male diets from three times during the pre-study and study interval concurrent with the concentration analyses. Aliquots were taken from six locations in each container sampled. Results of analyses demonstrated that the test material was homogeneously distributed within the feed with low mean relative standard deviations in the range of 1.82-6.24%.

Stability Analysis: (Results are from the 4-week mouse dietary study, Study No. 001048). The actual concentration of XDE-750 in the 10 and 1000 mg/kg bw/day test diets, expressed as percentage of the nominal concentration, were as follows:

Dose (mg/kg bw/day)		
Storage Interval	**10**	**1000**
Day 0	95.6%	109.0%
Day 21	100.6%	116.8%

Concentration Analysis: Analysis: The range of values for the actual concentrations of XDE-750 in the test diets, and the overall mean values, expressed as percentage of the nominal concentrations, were as follows:

Dose (mg/kg bw/day)					
	0	**10**	**100**	**500**	**1000**
Actual concentration					
Range of values	None detected	9.9 to 12.6	78 to 114	460 to 540	890 to 1170
Mean value		11.5	93	513	1000
% of target concentration					
Range of values	None detected	99% to 126%	78% to 114%	92% to 108%	89% to 117%
Mean value		115%	93%	103%	100%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics: Means and standard deviations were calculated for all continuous data. Body weights, food consumption, organ weights, appropriate hematologic data and clinical chemistry data were evaluated by Bartlett's test for equality of variances, alpha=0.01. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance (ANOVA). If significant at alpha=0.05, the ANOVA were followed respectively by Dunnett's test, alpha=0.05 or the Wilcoxon Rank-Sum test, alpha=0.05, with a Bonferroni correction for multiple comparisons to the control. The experiment-wise alpha level was reported for these 2 tests. DCO incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group, alpha=0.05. Data collected at different time points were analyzed separately. Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices and differential WBC counts. Statistical outliers were identified by a sequential test, alpha=0.05, but routinely excluded only from food consumption calculations. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons.
Because numerous measurements were statistically compared to the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

C. METHODS:

1. Observations: Twice daily a cage-side examination was conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Detailed clinical observations (DCO) were conducted on a weekly basis, and included cage-side, hand-held and open-field observations.

2. Body Weight: Individual body weights were measured on a weekly basis throughout the study period.

3. Food Consumption and Compound Intake: Food consumption was measured once a week throughout the study period. From the body weight and food intake data, the actual test material intake per animal (mg/kg bw/day) was calculated.

4. Ophthalmoscopic Examination: Eyes of all animals were examined prior to study initiation and during the week prior to necropsy using indirect ophthalmoscopy, and during necropsy using a moistened glass slide pressed to the cornea.

5. Hematology & Clinical Chemistry: Blood samples were taken on the day of necropsy from all animals, anesthetized with carbon dioxide, via the orbital sinus, for hematology and clinical chemistry analysis. Animals were not fasted prior to collection. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

40

a. Hematology:

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
✓	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium		Globulins
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (ALT/also SGPT)		
✓	Serum aspartate amino-transferase (AST/also SGOT)		
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis: Not conducted.

7. Sacrifice and Pathology: At study termination, animals were anesthetized by inhalation of carbon dioxide, sacrificed by decapitation, and then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Oral tissues	✓	Aorta	✓	Brain
✓	Salivary glands	✓	Heart	✓	Periph.nerve
✓	Esophagus	✓	Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)

✓	Jejunum	✓	Thymus		**GLANDULAR**
		✓			
✓	Ileum			✓	Adrenal gland
				✓	
✓	Cecum		**UROGENITAL**	✓	Lacrimal/Harderian glands
✓	Colon	✓	Kidneys	✓	Mammary gland
		✓			
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
✓✓	Liver	✓	Testes	✓	Thyroids
		✓			
✓	Gall bladder	✓	Epididymides		Auditory sebaceous glands
		✓			
✓	Pancreas	✓	Prostate	✓	Coagulating glands
	RESPIRATORY	✓	Seminal vesicle		**OTHER**
✓	Trachea	✓	Ovaries and oviducts	✓	Bone
		✓			
✓	Lung	✓	Uterus	✓	Skeletal muscle
		✓			
✓	Nasal tissues	✓	Cervix	✓	Skin
	Pharynx	✓	Vagina	✓	All gross lesions and masses
✓	Larynx				

The (✓) tissues were examined from all animals in the control and 1000 mg/kg bw/day groups. In addition, liver, lungs, kidneys, relevant gross lesions and target organs were examined for all animals in the 10, 100 and 500 mg/kg bw/day groups.

II. RESULTS

A. Observations:

1. **Mortality:** All animals survived the duration of the study period.

2. **Clinical Observations:** There were no overt, clinical signs of treatment-related toxicity.

B. Body Weight and Weight Gain: There was no treatment-related effect on body weight or body weight gain.

C. Food Consumption and Compound Intake:

1. **Food Consumption:** There were no treatment-related findings.

2. **Compound Consumption:** Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

3. **Food Efficiency:** Not conducted.

D. Ophthalmoscopic Examination: There were no treatment-related findings.

E. Blood Analyses:

1. Hematology: There were no treatment-related findings.

2. Clinical Chemistry: There were no treatment-related findings.

F. Urinalysis: Not conducted.

G. Sacrifice and Pathology:

1. Organ Weight: Refer to Table 2. Testes and epididymides weights were decreased for males in the 1000 mg/kg bw/day group. However, this finding was not statistically significant, there was no dose-response relationship and there were no corresponding histopathological findings. Hence, the noted decreases in testes and epididymides weights were not considered to be treatment-related.

TABLE 2 - Testes and Epididymides Weights of Males [a], absolute (g) and relative to bw (g/100 g)

	Dose (mg/kg bw/day)				
	0	10	100	500	1000
Testes - absolute	0.257±0.037	0.258±0.020	0.245±0.028	0.250±0.030	0.240±0.031
- relative	0.696±0.100	0.694±0.084	0.668±0.095	0.651±0.104	0.608±0.120
Epididymides - absolute	0.109±0.012	0.111±0.013	0.112±0.010	0.112±0.014	0.103±0.013
- relative	0.295±0.026	0.299±0.036	0.305±0.027	0.292±0.044	0.259±0.046

[a] Data obtained from pages 59 and 60 in the study report, n=10.

2. Gross Pathology: There were no treatment-related findings.

3. Microscopic Pathology: There were no treatment-related findings.

III. DISCUSSION

A. Investigators' Conclusions: "There were no treatment-related effects in any of the parameters. Based on the multiple parameters evaluated in this study, the no-observed-effect level (NOEL) for CD-1 mice of either sex was the limit test dose of 1000 mg/kg/day XDE-750."

B. Reviewer Comments: Male and female CD-1 mice were fed test diets containing technical XDE-750, purity 94.5%, at dose levels of 0, 10, 100, 500 or 1000 mg/kg bw/day (equal to 0, 10.2, 101, 512 or 1020 mg/kg bw/day for males, and 0, 102, 103, 515 or 1020 mg/kg bw/day for females) for a period of 13 weeks, 10 mice per sex per group. There were no treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, organ weights, hematology, clinical

43

chemistry, gross pathology or histopathology.
Since there were no treatment-related findings observed for any of the parameters evaluated in this study, the LOAEL could not be determined. The NOAEL is 1000 mg/kg bw/day (equal to 1020.0/1020.0 mg/kg bw/day).

C. **Study Deficiencies:** No scientific deficiencies were noted in the study.

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** for
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Repeat-dose Feeding Toxicity Study in Mice; OECD 407.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 95.4% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Yano, B.L., et al (2000) XDE-750: 4-Week Repeated Dose Dietary Toxicity Study in CD-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 001048. August 2, 2000. Unpublished

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a 4 week repeat-dose feeding toxicity study (MRID 46235624), technical XDE-750, purity 95.4%, was administered to 5 CD-1 mice per sex per group in the diet at dose levels of 0, 10, 100, 500 or 1000 mg/kg bw/day (equal to 0, 11.0, 102.0, 524.7 or 1038.0 mg/kg bw/day for males, and 0, 10.8, 105.0, 530.4 or 1058.0 mg/kg bw/day for females). There were no adverse, treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, organ weights, hematology, clinical chemistry, urinalysis or histopathology.
WBC count was lower in the 1000 mg/kg bw/day group, both sexes, without corresponding histopathological changes. This finding could possibly represent an increase in the destruction of circulating WBCs, and so is considered to be an adverse effect. Histopathological findings considered to be treatment-related were limited to 2 males in the high-dose group, manifest as a generalized decrease in hepatocyte glycogen, and hepatocyte hypertrophy with altered tinctorial properties. These effects were not considered adverse in the absence of any corresponding clinical chemistry of histopathological findings or liver weight changes.

The LOAEL was 1000 mg/kg bw/day (1038.0/1058.0 mg/kg bw/day) based on a decrease in WBC count. The NOAEL is 500 mg/kg bw/day (524.7/530.3 mg/kg bw/day) .

This subchronic toxicity study in the rat is acceptable and satisfies the guideline requirement (OECD 407) for a repeat-dose oral study in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

45

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-125; TSN102095
Purity:	95.4% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5002 Certified Rodent Lab Diet).

3 **Test Animals:**

Species:	Mouse	
Strain:	CD-1	
Age/weight at study initiation:	7 weeks of age; Males, 26.3 g to 33.6 g; Females, 22.5 g to 26.0 g.	
Source:	Charles River Laboratories, Inc., Portage, MI.	
Housing:	Individually housed in suspended stainless steel cages with wire-mesh floors.	
Diet:	Purina Certified Rodent Lab Diet #5002 in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	40-70%
	Air changes:	12-15/hr
	Photoperiod:	12hrs dark/12 hrs light
Acclimation period:	7 days.	

B. STUDY DESIGN:

1. **In Life Dates:** April 18, 2000 to May 17, 2000.

2. **Animal Assignment:** Animals were stratified by body weight and then randomly assigned to the test groups noted in Table 1 using a computer program.

TABLE 1 - Study Design

Test Group	Conc. in Diet (mg/kg bw/day)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	37986	5	5
Low	10	11.0/10.8	5	5
Mid-low	100	102.0/105.0	5	5
Mid-high	500	524.7/530.4	5	5
High	1000	1038.0/1058.0	5	5

The high dose level of 1000 mg/kg bw/day represented the limit dose. The remaining dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group and to ensure definition of a no-observed-effect level (NOEL).

3. **Diet Preparation and Analysis:** Fresh diets were prepared weekly based on the most recent body weight and food consumption data. Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Stability of the test material in diet for up to 21 days was determined prior to study initiation at dose levels of 10 mg/kg bw/day (female diet) and 1000 mg/kg bw/day (male diet). Homogeneity of mixing was not determined. Actual test material concentration in the diet was determined for all dose levels from test diet prepared just prior to study initiation.

Results - Homogeneity Analysis: Not conducted.

Stability Analysis: The actual concentration of XDE-750 in the 10 and 1000 mg/kg bw/day test diets, expressed as percentage of the nominal concentration, were as follows:

Dose (mg/kg bw/day)		
Storage Interval	**10**	**1000**
Day 0	95.6%	109.0%
Day 21	100.6%	116.8%

Concentration Analysis: Individual samples of test diets at all dose levels ranged from 95% to 104% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Means and standard deviations were calculated for all continuous data. All parameters examined statistically were first evaluated by Bartlett's test for equality of variances. If the results from Bartlett's test were significant at alpha = 0.01, then the data for the parameter may have been subjected to a transformation to obtain equality of the variances. The transformations that were examined were the common log, the inverse and the square root. The data were reviewed and an appropriate form of the data was selected. The selected form of the data was then subjected to the appropriate parametric analysis as described below.
In-life body weights were evaluated using a repeated measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex and dose. In the RM-ANOVA, differences between the groups were detected primarily by the time-dose interaction.
The first examination in the RM-ANOVA was the time-sex-dose interaction. If significant at alpha = 0.02, the analysis was repeated separately for each sex without examining the results of other factors. The time-dose interaction was examined next at alpha = 0.05. If the time-dose interaction was statistically identified, linear contrasts tested the time-dose interaction for the comparisons of each dose group to the control group. A Bonferroni correction was applied to the alpha level to compensate for the multiple comparisons with the control group. This correction controls the experiment-wise error rate. The corrected comparison-wise alpha level of 0.02 was reported so direct comparison could be made to the p-values generated.
Terminal body weight and organ weight (absolute and relative, excluding epididymides and testes), haematologic parameters (excluding RBC indices and differential WBC) and clinical chemistry parameters were evaluated using a two-way ANOVA with the factors of sex and dose. Differences between the groups were primarily detected by the dose factor. For these parameters the first examination was whether the sex-dose interaction was significant at alpha = 0.05; if it was, a one-way ANOVA was done separately for each sex. Comparisons of individual dose groups to the control group were made with a Dunnett's test only when a statistically significant dose effect existed (alpha = 0.05). Dunnett's

test corrects for multiple comparisons to the control and the experiment-wise error rate was reported. Epididymides and testes weights were analyzed using a one-way ANOVA. If significant dose effects (alpha = 0.05) were determined in the one-way ANOVA then separate doses were compared to controls using a Dunnett's test.
Food consumption data were evaluated by Bartlett's test for equality of variances. Exploratory data analysis was performed by a parametric analysis of variance (ANOVA) and if significant at alpha = 0.05, followed by Dunnett's test, alpha = 0.05. The experiment-wise alpha level was reported.
Descriptive statistics only were reported for body weight gains, RBC indices and differential WBC counts. Statistical outliers were identified by a sequential test, and routinely excluded from food consumption statistics. Other outliers may have been excluded only for documented, scientifically sound reasons. DCO incidence scores were qualitatively evaluated.
Because numerous measurements were statistically compared to the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results are consistent with other biological and pathological findings and historical control values.

C. METHODS:

1. Observations: Twice daily a cage-side examination was conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Detailed clinical observations (DCO) were conducted on a weekly basis, and included cage-side, hand-held and open-field observations.

2. Body Weight: Individual body weights were measured twice during the first week of the study, and once a week thereafter.

3. Food Consumption and Compound Intake: Food consumption was measured twice during the first week of the study, and once a week thereafter. From the body weight and food intake data, the actual test material intake per animal (mg/kg bw/day) was calculated.

4. Ophthalmoscopic Examination: Eyes of all animals were examined prior to study initiation and prior to termination using indirect ophthalmoscopy, and during necropsy using a moistened glass slide pressed to the cornea.

5. Hematology & Clinical Chemistry: Blood samples were taken on the day of necropsy from all animals, while under methoxyflurane anesthesia, via the orbital sinus, for hematology and clinical chemistry analysis. Animals were not fasted prior to collection. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

a. Hematology:

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
	Blood clotting measurements		Reticulocyte count
✓	(Platelet count)		
	(Thromboplastin time)		

	(Clotting time) (Prothrombin time)		

b. **Clinical Chemistry:**

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium		Globulins
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (ALT/also SGPT)		
✓	Serum aspartate amino-transferase (AST/also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Ornithine decarboxylase		

6. **Urinalysis:** Not conducted.

7. **Sacrifice and Pathology:** At study termination, animals were anesthetized by inhalation of methoxyfluorane, sacrificed by decapitation, and then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Oral tissues	✓	Aorta	✓	Brain
				✓	
✓	Salivary glands	✓	Heart	✓	Periph.nerve
		✓			
✓	Esophagus	✓	Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)
		✓			
✓	Jejunum	✓	Thymus		**GLANDULAR**
		✓			
✓	Ileum			✓	Adrenal gland
				✓	
✓	Cecum		**UROGENITAL**	✓	Lacrimal/Harderian glands
✓	Colon	✓	Kidneys	✓	Mammary gland
		✓			
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
✓✓	Liver	✓	Testes	✓	Thyroids
		✓			
✓	Gall bladder	✓	Epididymides		Auditory sebaceous glands
		✓			
✓	Pancreas	✓	Prostate	✓	Coagulating glands

	RESPIRATORY	✓	Seminal vesicle		OTHER
✓	Trachea	✓	Ovaries and oviducts	✓	Bone
✓	Lung	✓	Uterus	✓	Skeletal muscle
✓	Nasal tissues	✓	Cervix	✓	Skin
	Pharynx	✓	Vagina	✓	All gross lesions and masses
✓	Larynx				

The (✓) tissues were examined from all animals in the control and 1000 mg/kg bw/day groups. In addition, liver, kidneys and relevant gross lesions were examined for all animals in the 10, 100 and 500 mg/kg bw/day groups.

II. RESULTS

A. Observations

1. **Mortality**: All animals survived the duration of the study period.

2. **Clinical Observations**: There were no overt, clinical signs of treatment-related toxicity.

B. Body Weight and Weight Gain: There was no treatment-related effect on body weight or body weight gain.

C. Food Consumption and Compound Intake:

1. **Food Consumption**: There were no treatment-related findings.

2. **Compound Consumption**: Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

3. **Food Efficiency**: Not conducted.

D. Ophthalmoscopic Examination: There were no treatment-related findings.

E. Blood Analyses:

1. **Hematology**: Refer to Table 2. White blood cell count was decreased in the 1000 mg/kg bw/day group, both sexes, falling outside of the historical control range of values for males only. The study authors did not consider this to be a toxicologically significant finding since there were no corresponding histopathological findings in the bone marrow (e.g., decreased production). However, an increase in the destruction of circulating WBCs (e.g., without any histopathological changes to the bone marrow) resulting in a decrease in WBC count would be considered an adverse effect. Hence, the PMRA reviewer considers this finding to possibly be toxicologically significant. The longer term mouse studies will aid in determining the significance of these noted effects.

TABLE 2 - WBC Counts for Males and Females[a], $E^3/\mu L$

	Dose (mg/kg bw/day)					
	0	10	100	500	1000	Historical control data[b]
Males	6.37±3.91	4.13±1.38	4.93±3.19	4.17±1.44	2.90±1.17*	3.76-9.02
Females	5.79±1.23	4.26±1.14	4.74±2.07	5.27±1.11	3.67±0.85*	2.67-6.39

[a] Data obtained from pages 51 and 53 in the study report; n=5.
[b] Historical control group mean range obtained from six, 4-week dietary studies conducted since 1997; individual range of values was not provided.
* statistically significantly different from control, $p < 0.05$

2. **Clinical Chemistry**: There were no treatment-related findings.

F. **Urinalysis**: Not conducted.

G. **Sacrifice and Pathology**:

1. **Organ Weight**: Refer to Table 3. Adrenal weight was increased for males in the 1000 mg/kg bw/day group. However, this finding was not statistically significant, there was no dose-response relationship and there were no corresponding histopathological findings. Hence, the noted increase in adrenal weight was not considered to be treatment-related.

TABLE 3 - Adrenal Weights of Males [a], absolute (g) and relative to bw (g/100 g)

	Dose (mg/kg bw/day)				
	0	10	100	500	1000
Adrenal - absolute	0.0043±0.0008	0.0047±0.0004	0.0044±0.0009	0.0047±0.0003	0.0053±0.0010
- relative	0.0128±0.0026	0.0136±0.0015	0.0128±0.0024	0.0134±0.0010	0.0151±0.0025

[a] Data obtained from page 59 in the study report; n=5.

2. **Gross Pathology**: There were no treatment-related findings.

3. **Microscopic Pathology**: Refer to Table 4. The only findings considered to be treatment-related by the study author were observed in the liver of 2 males in the 1000 mg/kg bw/day group, manifest as a generalized decrease in hepatocyte glycogen, and hepatocyte hypertrophy with altered tinctorial properties.

TABLE 4 - Selected Liver Histopathology in Male Mice [a]

	Dose (mg/kg bw/day)				
	0	10	100	500	1000
Decreased hepatocyte glycogen	0	1	1	0	2
Hepatocyte hypertrophy, altered tinctorial properties	0	0	0	0	2

[a] Data obtained from page 74 in the study report; n=5.

III. DISCUSSION

A. Investigators' Conclusions: "Male and female mice were given 0, 10, 100, 500 or 1000 mg/kg/day for 4 weeks. Treatment-related effects were limited to two of five male mice given 1000 mg/kg/day and consisted of an increase in hepatocyte size with altered cytoplasmic staining of the cytoplasm, and a decrease in glycogen. The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg/day for males and females. The no-observed-effect level (NOEL) was determined to be 500 mg/kg/day for males and 1000 mg/kg/day for females."

B. Reviewer Comments: Male and female CD-1 mice were fed test diets containing technical XDE-750, purity 95.4%, at dose levels of 0, 10, 100, 500 or 1000 mg/kg bw/day (equal to 0, 11.0, 102.0, 524.7 or 1038.0 mg/kg bw/day for males, and 0, 10.8, 105.0, 530.4 or 1058.0 mg/kg bw/day for females) for a period of 28 days, 5 mice per sex per group. There were no treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, organ weights or clinical chemistry. WBC count was lower in the 1000 mg/kg bw/day group, both sexes, without corresponding histopathological changes. This finding could possibly represent an increase in the destruction of circulating WBCs, and so is considered to be an adverse effect. Histopathological findings considered to be treatment-related were limited to 2 males in the high-dose group, manifest as generalized decrease in hepatocyte glycogen, and hepatocyte hypertrophy with altered tinctorial properties. These effects were not considered adverse in the absence of any corresponding clinical chemistry findings, histopathology or liver weight changes.
Based on the results of this study, the LOAEL was 1000 mg/kg bw/day (equal to 1038.0/1058.0 mg/kg bw/day) based on a decrease in WBC count. The NOAEL is 500 mg/kg bw/day (equal to 524.7/530.4 mg/kg bw/day).

C. Study Deficiencies: No scientific deficiencies were noted in the study.

57

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** for
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Study in Rats; OPPTS 870.3100 (rodent); OECD 408.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Dryzga, M.; Stebbins, K. (2001) Revised Report for XDE-750: 13-Week Dietary Toxicity with 4-Week Recovery Study in Fischer 344 Rats. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 001221, May 17, 2001. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 46235621), XDE-750, purity 94.5%, was administered to 10 Fischer 344 rats/sex/dose in the diet at dose levels of 0, 10, 100, 500 and 1000 mg/kg bw/day (equal to 0, 10.9, 109, 543 and 1090 mg/kg bw/day for males, and 0, 10.7, 108, 540 and 1060 mg/kg bw/day for females). An additional 10 rats per sex were assigned to the 0 and 1000 mg/kg bw/day groups (equal to 1120/1030 mg/kg bw/day) for a 4-week recovery period following treatment. ^13 weeks
There were no adverse, treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, hematology, clinical chemistry or urinalysis. Full cecal weights were increased in the 500 and 1000 mg/kg bw/day groups, both sexes, and empty cecal weights were increased in the 1000 mg/kg bw/day group, both sexes, and in the 500 mg/kg bw/day group, males only. At gross pathological examination, there was an increase in the size of the cecum for all animals in the 1000 mg/kg bw/day group. Histopathological examination revealed hyperplasia of the mucosal epithelium of the cecum and ileum in the 1000 mg/kg bw/day group, males only.
Following the 4-week recovery period, there was complete recovery of the hyperplasia of the mucosal epithelium of the cecum and ileum, and partial recovery of the increased cecal weights in the 1000 mg/kg bw/day group.
For males, the LOAEL is 1000 mg/kg bw/day (1090 mg/kg bw/day), based on hyperplasia of the mucosal epithelium of the cecum and ileum. The NOAEL is 500 mg/kg bw/day (543 mg/kg bw/day).
For females, the LOAEL cannot be determined since there were no adverse treatment-related findings observed at any dose level tested. The increased size of the cecum and increased full cecal weights observed in the 1000 mg/kg bw/day group were not considered adverse effects since there

were no corresponding histopathological findings. The NOAEL is 1000 mg/kg bw/day (1060 mg/kg bw/day).

This subchronic toxicity study in the rat is acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-143; TSN102319
Purity:	94.5% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5002 Certified Rodent Diet).

3. **Test Animals:**

Species:	Rat	
Strain:	Fischer 344	
Age/weight at study initiation:	6 weeks of age; Males, 122.3 g to 138.8 g; Females, 96.4 g to 110.2 g.	
Source:	Charles River Laboratories Inc., Raleigh, NC.	
Housing:	Individually housed in suspended stainless steel cages with wire-mesh floors.	
Diet:	Purina Certified Rodent Lab Diet #5002 in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	40-70%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	One week	

B. STUDY DESIGN:

1. **In Life Dates:** November 1, 2000 to March 1, 2001.

2. **Animal Assignment:** Animals were stratified by body weight and then randomly assigned using a computer program to the test groups noted in Tables 1a and 1b.

TABLE 1a - Study Design, 13-Week Study

Test Group	Conc. in Diet (mg/kg bw/day)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	0/0	10	10

Low	10	10.9/10.7	10	10
Mid-low	100	109/108	10	10
Mid-high	500	543/540	10	10
High	1000	1090/1060	10	10

TABLE 1b - Study Design, 4-Week Recovery

Test Group	Conc. in Diet (mg/kg bw/day)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	0/0	10	10
High	1000	1120/1030	10	10

The high-dose level of 1000 mg/kg bw/day represented the limit dose level and was chosen based on the results of a 4-week dietary study. The high-dose was expected to produce some increased size of the cecum. The remaining dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group and to ensure definition of a no-observed-effect level (NOEL).

3. **Diet Preparation and Analysis:** Fresh diets were prepared weekly based on the most recent body weight and food consumption data. Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Stability of the test material in diet for up to 21 days was demonstrated in the 4-week dietary toxicity study in mice (Study No. 001048), and so stability analyses were not conducted in the 4-week rat study. Homogeneity of mixing was determined for the low-dose female diets and high-dose male diets prior to study initiation, approximately mid-way through the study and near study termination. Actual test material concentration in the diet was determined for all dose levels from test diets prepared just prior to study initiation, approximately mid-way through the study and near the end of the study.

Results - Homogeneity Analysis: Individual samples of 3 separate batches of the 10 mg/kg bw/day female test diets ranged from 100.6% to 115.7%, 91.7% to 112.9% 82.0% to 95.2% of the nominal concentration, respectively.

Individual samples of 3 separate batches of the 1000 mg/kg bw/day male test diets ranged from 95.2% to 101.9%, 71.2% to 98.8% and 71.3% to 101.5% of the nominal concentration, respectively.

Stability Analysis: (Results are from the 4-week mouse dietary study, Study No. 001048). The actual concentration of XDE-750 in the 10 and 1000 mg/kg bw/day test diets, expressed as percentage of the nominal concentration, were as follows:

Dose (mg/kg bw/day)		
Storage Interval	10	1000
Day 0	95.6%	109.0%
Day 21	100.6%	116.8%

Concentration Analysis: Individual samples of test diets at all dose levels ranged from 89% to 113% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Means and standard deviations were calculated for all continuous data. Body weights, food consumption, urine volume, urine specific gravity, appropriate hematologic data and clinical chemistry data were evaluated by Bartlett's test for equality of variances, alpha=0.01. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance (ANOVA). If significant at alpha=0.05, the ANOVA were followed respectively by Dunnett's test, alpha=0.05 or the Wilcoxon Rank-Sum test, alpha=0.05 with a Bonferroni correction for multiple comparisons to the control. The experiment-wise alpha level was reported for these 2 tests. DCO incidence scores were statistically analyzed by a z-test of proportions comparing each treated group tp the control group, alpha=0.05. Data collected at different time points were analyzed separately. Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices and differential WBC counts. Statistical outliers were identified by a sequential test, alpha=0.05, but routinely excluded only from food consumption calculations. Outliers may have been excluded frm other analyses only for documented, scientifically sound reasons.
Because numerous measurements were statistically compared to the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

C. METHODS:

1. **Observations:** Twice daily a cage-side examination was conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Detailed clinical observations (DCO) were conducted on a weekly basis, and included cage-side, hand-held and open-field observations.

2. **Body Weight:** Individual body weights were measured on a weekly basis throughout the study period.

3. **Food Consumption and Compound Intake:** Food consumption was measured once a week throughout the study period. From the body weight and food intake data, the actual test material intake per animal (mg/kg bw/day) was calculated.

4. **Ophthalmoscopic Examination:** Eyes of all animals were examined prior to study initiation and during the week prior to necropsy using indirect ophthalmoscopy, and during necropsy using a moistened glass slide pressed to the cornea.

5. **Hematology & Clinical Chemistry:** Blood samples were taken on the day of necropsy from all animals via the orbital sinus, for hematology and clinical chemistry analysis. Animals were fasted prior to collection, and were anesthetized with carbon dioxide. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

a. **Hematology**:

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
✓	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
✓	(Prothrombin time)		

b. **Clinical Chemistry**:

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium		Globulins
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (ALT/also SGPT)		
✓	Serum aspartate amino-transferase (AST/also SGOT)		
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. **Urinalysis**: Urine samples were collected from all nonfasted, main group, animals during the week *prior to necropsy using timed urine volume collection, as well as by manual compression of the urinary* bladder. The CHECKED (✓) parameters were examined.

✓	Appearance	✓	Glucose
	Volume	✓	Ketones
✓	Specific gravity	✓	Bilirubin
✓	pH	✓	Blood
✓	Sediment (microscopic)		Nitrate
✓	Protein	✓	Urobilinogen

7. **Sacrifice and Pathology**: At study termination, animals were anesthetized by inhalation of carbon dioxide, sacrificed by decapitation, and then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Tongue and oral tissues	✓	Aorta	✓	Brain
				✓	
✓	Salivary glands	✓	Heart	✓	Periph.nerve
		✓			
✓	Esophagus	✓	Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)
		✓			
✓	*Jejunum*	✓	Thymus		GLANDULAR
		✓			
✓	Ileum			✓	Adrenal gland
				✓	
✓✓	Cecum		UROGENITAL	✓	Lacrimal/Harderian glands
✓	Colon	✓	Kidneys	✓	Mammary gland
		✓			
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
✓✓	Liver	✓	Testes	✓	Thyroids
		✓			
	Gall bladder	✓	Epididymides	✓	Auditory sebaceous glands
		✓			
✓	Pancreas	✓	Prostate	✓	Coagulating glands
	RESPIRATORY	✓	Seminal vesicle		OTHER
✓	Trachea	✓	Ovaries and oviducts	✓	Bone
		✓			
✓	Lung	✓	Uterus	✓	Skeletal muscle
		✓			
✓	Nasal tissue	✓	Cervix	✓	Skin
	Pharynx	✓	Vagina	✓	All gross lesions and masses
✓	Larynx				

The (✓) tissues were examined from all animals in the control and 1000 mg/kg bw/day groups. In addition, the lungs, liver, kidneys, cecum, ileum and relevant gross lesions were examined for all animals in the 10, 100 and 500 mg/kg bw/day groups.

II. RESULTS

A. Observations

1. **Mortality:** All animals survived the duration of the study period.

2. **Clinical Observations:** There were no overt, clinical signs of treatment-related toxicity.

B. Body Weight and Weight Gain: There was no treatment-related effect on body weight or body weight gain.

C. Food Consumption and Compound Intake:

1. **Food Consumption:** There were no treatment-related findings.

2. **Compound Consumption:** Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

3. **Food Efficiency**: Not conducted.

D. **Ophthalmoscopic Examination:** There were no treatment-related findings.

E. **Blood analyses**:

1. **Hematology**: There were no treatment-related findings.

2. **Clinical Chemistry**: There were no treatment-related findings.

F. **Urinalysis:** There was a slight decrease in urinary pH in the 500 and 1000 mg/kg bw/day groups, both sexes, and a decrease in urinary protein and ketones in the 1000 mg/kg bw/day group, both sexes. These findings were considered to possibly be related to treatment, but were not considered adverse since there were no corresponding clinical chemistry or histopathological findings.

G. **Sacrifice and Pathology**

1. **Organ Weight:** i) Males: Refer to Tables 2 and 3. At the end of the 13-week treatment period, full and empty cecal weights were increased in the 500 and 1000 mg/kg bw/day groups. Partial recovery of cecal weights in the 1000 mg/kg bw/day group was observed at the end of the 4-week recovery period. The only other finding was decreased thymus weight noted at all dose levels, and attaining statistical significance for absolute weights in the 10, 500 and 1000 mg/kg bw/day groups. However, there was no dose-response relationship, relative weights were not statistically significant, there were no corresponding histopathological findings and all thymus weights fell within the historical control range of values. Hence, the decrease in thymus weights was not considered to be treatment-related.

TABLE 2 - Full and Empty Cecal Weights of Males [a], absolute (g) and relative to bw (g/100 g)

	Dose mg/kg bw/day)				
	0	10	100	500	1000
Main group					
Full cecum - absolute	4.720±0.435	4.542±0.457	4.953±0.640	8.838±1.386*	13.289±1.380*
- relative	1.532±0.178	1.476±0.145	1.603±0.214	2.899±0.462*	4.406±0.517*
Empty cecum- absolute	1.831±0.246	1.754±0.144	1.780±0.196	2.080±0.268*	2.361±0.124*
- relative	0.590±0.056	0.570±0.039	0.576±0.054	0.680±0.069*	0.781±0.030*
Recovery group					
Full cecum - absolute	4.062±0.788	---	---	---	6.418±1.208*
- relative	1.244±0.244	---	---	---	2.028±0.336*
Empty cecum- absolute	1.510±0.162	---	---	---	1.815±0.253*
- relative	0.463±0.039	---	---	---	0.573±0.055*

[a] Data obtained from pages 143 and 147 in the study report; n=10.

* statistically significantly different from control, $p < 0.05$.

TABLE 3 - Thymus Weights of Males [a], absolute (g) and relative to bw (g/100 g)

	Dose (mg/kg bw/day)					
	0	10	100	500	1000	Historical control data[b]
Thymus - absolute	0.235±0.029	0.209±0.013*	0.215±0.026	0.208±0.024*	0.204±0.017*	0.17-0.255
- relative	0.076±0.009	0.068±0.005	0.069±0.007	0.068±0.010	0.068±0.004	0.059-0.085

[a] Data obtained from page 143 in the study report; n=10.
[b] Historical control group mean range obtained from three, 13-week dietary studies conducted since 1997; individual range of values was not provided.
* statistically significantly different from control, $p < 0.05$

ii) Females: Refer to Table 4. At the end of the 13-week treatment period, full cecal weights were increased in the 500 and 1000 mg/kg bw/day groups, and empty cecal weights were increased in the 1000 mg/kg bw/day group. Partial recovery of cecal weights in the 1000 mg/kg bw/day group was observed at the end of the 4-week recovery period. There were no other treatment-related findings.

TABLE 4 - Full and Empty Cecal Weights of Females [a], absolute (g) and relative to bw (g/100 g)

	Dose mg/kg bw/day)				
	0	10	100	500	1000
Main group					
Full cecum - absolute	3.450±0.331	3.441±0.397	3.660±0.482	4.801±0.825*	9.451±1.090*
- relative	2.022±0.204	2.021±0.215	2.141±0.228	2.831±0.492	5.594±0.511*
Empty cecum- absolute	1.167±0.168	1.184±0.134	1.175±0.090	1.237±0.109	1.539±0.169*
- relative	0.684±0.102	0.695±0.067	0.688±0.040	0.729±0.063	0.910±0.060*
Recovery group					
Full cecum - absolute	3.417±0.454	---	---	---	5.265±0.661*
- relative	2.001±0.373	---	---	---	3.033±0.364*
Empty cecum- absolute	1.133±0.103	---	---	---	1.379±0.167*
- relative	0.661±0.080	---	---	---	0.807±0.060*

[a] Data obtained from pages 146 and 148 in the study report; n=10.
* statistically significantly different from control, $p < 0.05$

2. **Gross Pathology**: Refer to Table 4. After 13 weeks of treatment, all animals in the 1000 mg/kg bw/day exhibited increased size of the cecum, whereas at the end of the 4-week recovery period, only 2/10 males and 2/10 females were affected, indicating partial recovery. There were no other treatment-related findings.

Table 3 - Selected Gross Pathological Findings in the Cecum[a]

	Dose, mg/kg bw/day				
Increased size	**0**	**10**	**100**	**500**	**1000**
At 13-weeks:					
Males	0	0	0	0	10
Females	0	0	0	0	10
After 4 weeks recovery:					
Males	0	0	0	0	2
Females	0	0	0	0	2

[a] Data obtained from pages 149, 150, 157 and 158 in the study report; n=10.

3. **Microscopic Pathology:** Refer to Table 4. After 13 weeks of treatment, all males in the 1000 mg/kg bw/day group exhibited slight diffuse hyperplasia of the mucosal epithelium of the ileum and cecum. There were no other findings considered to be related to treatment with XDE-750.
After the 4-week recovery period, there were no treatment-related histopathological findings, indicating complete recovery of the epithelial hyperplasia of the cecum and ileum noted for males in the 1000 mg/kg bw/day group at the end of the 13-week treatment period.

Table 4 - Selected Histopathological Findings in the Cecum and Ileum of Male Rats[a]

	Dose, mg/kg bw/day				
Epithelial hyperplasia, diffuse, very slight	**0**	**10**	**100**	**500**	**1000**
Cecum					
Males	0	0	0	0	10
Females	0	0	0	0	0
Ileum					
Males	0	0	0	0	10
Females	0	0	0	0	0

[a] Data obtained from page 166 in the study report; n=10.

III. DISCUSSION

A. **Investigators' Conclusions:** "After 13 weeks of dosing, males given 1000 mg/kg/day had treatment-related hyperplasia of the mucosal epithelium of the cecum and ileum. The hyperplasia of the cecal epithelium corresponded to a statistically-identified increase in mean cecal weight for males given 1000 mg/kg/day. Males given 500 mg/kg/day and females given 500 or 1000 mg/kg/day also had treatment-related increases in cecal weights; however, there were no corresponding microscopic alterations of the cecum or ileum in these animals. The only other treatment-related alterations were decreases in urine pH for males and females given 500 or 1000 mg/kg/day, and decreases in urine protein and ketones for males and females given 1000 mg/kg/day. Following a 4-week recovery period, there was complete resolution of the hyperplasia of the mucosal epithelium of the cecum and ileum and partial recovery of the increased cecal weights of males and females from the 1000 mg/kg/day group. In addition, there was complete recovery of the urine alterations in males and females from the 1000 mg/kg/day group. Based on changes in cecal weight and urine pH, the no-observed-effect level (NOEL) for male and female Fischer 344 rats was the targeted dietary dose of 100 mg/kg/day. At 500 mg/kg/day for males and 1000 mg/kg/day for

females, increases in cecal weights and decreases in urine pH were unaccompanied by microscopic alterations. Therefore, the no-observed-adverse-effect level (NOAEL) for males was 500 mg/kg/day, and the NOAEL for females was 1000 mg/kg/day."

B. Reviewer Comments: Male and female Fischer 344 rats were fed test diets containing technical XDE-750, purity 94.5%, at dose levels of 0, 10, 100, 500 or 1000 mg/kg bw/day (equal to 0, 10.9, 109, 543 or 1090 mg/kg bw/day for males, and 0, 10.7, 108, 540 or 1060 mg/kg bw/day for females) for a period of 13 weeks, 10 rats per sex per group. An additional 10 rats per sex were assigned to the 0 and 1000 mg/kg bw/day groups (equal to 1120/1030 mg/kg bw/day) for a 4-week recovery period following treatment. There were no adverse, treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, hematology, clinical chemistry or urinalysis. Full cecal weights were increased in the 500 and 1000 mg/kg bw/day groups, both sexes, and empty cecal weights were increased in the 1000 mg/kg bw/day group, both sexes, and in the 500 mg/kg bw/day group, males only. At gross pathological examination, there was an increase in the size of the cecum for all animals in the 1000 mg/kg bw/day group. Histopathological examination revealed hyperplasia of the mucosal epithelium of the cecum and ileum in the 1000 mg/kg bw/day group, males only.
Following the 4-week recovery period, there was complete recovery of the hyperplasia of the mucosal epithelium of the cecum and ileum, and partial recovery of the increased cecal weights in the 1000 mg/kg bw/day group.
Based on the results of this study, the LOAEL for males was determined to be 1000 mg/kg bw/day (equal to 1090 mg/kg bw/day) based on hyperplasia of the mucosal epithelium of the cecum and ileum. The NOAEL was 500 mg/kg bw/day (equal to 543 mg/kg bw/day). For females, the LOAEL could not be determined since there were no adverse, treatment-related findings. The increased size of the cecum and increased full cecal weights observed in the 1000 mg/kg bw/day group were not considered adverse effects since there were no corresponding histopathological findings. The NOAEL was 1000 mg/kg bw/day (equal to 1060 mg/kg bw/day).

C. **Study Deficiencies:** No scientific deficiencies were noted in the study.

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:**
Fungicide/Herbicide Toxicological Evaluation Section. Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Repeat-Dose Feeding Toxicity Study in Rats; OECD 407.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 95.4% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Stebbins, K.E., et al (2000) XDE-750: 4-Week Repeated Dose Dietary Toxicity Study in Fischer 344 Rats. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 001031, August 8, 2000. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a 4 week repeat-dose feeding toxicity study (MRID 46235625), technical XDE-750, purity 95.4%, was administered to 5 Fischer 344 rats per sex per group in the diet at dose levels of 0, 10, 100, 500 or 1000 mg/kg bw/day (equal to 0, 11.4, 113.7, 561.1 or 1125.6 mg/kg bw/day for males, and 0, 12.2, 110.2, 550.5 or 1093.7 mg/kg bw/day for females). There were no adverse, treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, organ weights, hematology, clinical chemistry, urinalysis or histopathology.
The size of the cecum was increased in the 500 and 1000 mg/kg/day groups, both sexes. Since there were no associated histolopathological changes, this was not considered to be an adverse effect, but rather reflective of physiological changes to the digestive tract following ingestion of XR-750.
The LOAEL could not be determined since there were no adverse, treatment-related findings. The NOAEL is 1000 mg/kg bw/day (1125.6/1093.7 mg/kg bw/day) .

This subchronic toxicity study in the rat is acceptable and satisfies the guideline requirement (OECD 407) for a repeat-dose oral study in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description:	Technical: tan powder.
Lot/Batch #:	F-0031-125; TSN102095
Purity:	95.4% a.i.
Compound Stability:	Not stated
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5002 Certified Rodent Lab Diet).

3 **Test Animals:**

Species:	Rat	
Strain:	Fischer 344	
Age/weight at study initiation:	7 weeks of age; Males, 133.8 g to 171.0 g; Females, 96.6 g to 108.6 g.	
Source:	Charles River Laboratories Inc., Raleigh, NC.	
Housing:	Individually housed in suspended stainless steel cages with wire-mesh floors.	
Diet:	Purina Certified Rodent Lab Diet #5002 in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	21.5-23.6°C
	Humidity:	43.8-55.4%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days.	

B. STUDY DESIGN:

1. **In Life Dates:** April 17, 2000 to May 16, 2000.

2. **Animal Assignment:** Animals were stratified by body weight and then randomly assigned using a computer program to the test groups noted in Table 1.

TABLE 1 - Study Design

Test Group	Conc. in Diet (mg/kg bw/day)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	37986	5	5
Low	10	11.4/12.2	5	5
Mid-low	100	113.7/110.2	5	5
Mid-high	500	561.1/550.5	5	5
High	1000	1125.6/1093.7	5	5

The high dose level of 1000 mg/kg bw/day represented the limit dose. The remaining dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group and to ensure definition of a no-observed-effect level (NOEL).

3. **Diet Preparation and Analysis:** Fresh diets were prepared weekly based on the most recent body weight and food consumption data. The high-dose diet was prepared by mixing test material with control diet to obtain the desired percent of test material in the diet. Mid-high, mid-low and low-dose diets were prepared by serially diluting the high-dose level diet with control diet. Stability of the test material in diet for up to 21 days was demonstrated in the 4-week dietary toxicity study in mice (Study No. 001048), and so stability analyses were not conducted in the 4-week rat study. Homogeneity of mixing was not determined. Actual test material concentration in the diet was determined for all dose levels from test diet prepared just prior to study initiation and during study week 3.

Results - Homogeneity Analysis: Not conducted.

Stability Analysis: (Results are from the 4-week mouse dietary study, Study No. 001048). The actual concentration of XDE-750 in the 10 and 1000 mg/kg bw/day test diets, expressed as percentage of the nominal concentration, were as follows:

Dose (mg/kg bw/day)		
Storage Interval	**10**	**1000**
Day 0	95.6%	109.0%
Day 21	100.6%	116.8%

Concentration Analysis: *Individual samples of test diets at all dose levels ranged from* 86% to 105% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Means and standard deviations were calculated for all continuous data. All parameters examined statistically were first evaluated by Bartlett's test for equality of variances. If the results from Bartlett's test were significant at alpha = 0.01, then the data for the parameter may have been subjected to a transformation to obtain equality of the variances. The transformations that were examined were the common log, the inverse and the square root. The data were reviewed and an appropriate form of the data was selected. The selected form of the data was then subjected to the appropriate parametric analysis as described below.
In-life body weights were evaluated using a repeated measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex and dose. In the RM-ANOVA, differences between the groups were detected primarily by the time-dose interaction.
The first examination in the RM-ANOVA was the time-sex-dose interaction. If significant at alpha = 0.02, the analysis was repeated separately for each sex without examining the results of other factors. The time-dose interaction was examined next at alpha = 0.05. If the time-dose interaction was statistically identified, linear contrasts tested the time-dose interaction for the comparisons of each dose group to the control group. A Bonferroni correction was applied to the alpha level to compensate for the multiple comparisons with the control group. This correction controls the experiment-wise error rate. The corrected comparison-wise alpha level of 0.02 was reported so direct comparison could be made to the p-values generated.
Terminal body weight and organ weight (absolute and relative, excluding epididymides and testes), urine volume, urine specific gravity, hematologic parameters (excluding RBC indices and differential WBC) and clinical chemistry parameters were evaluated using a two-way ANOVA with the factors of sex and

dose. Differences between the groups were primarily detected by the dose factor. For these parameters the first examination was whether the sex-dose interaction was significant at alpha = 0.05; if it was, a one-way ANOVA was done separately for each sex. Comparisons of individual dose groups to the control group were made with a Dunnett's test only when a statistically significant dose effect existed (alpha = 0.05). Dunnett's test corrects for multiple comparisons to the control and the experiment-wise error rate was reported.
Epididymides and testes weights were analyzed using a one-way ANOVA. If significant dose effects (alpha = 0.05) were determined in the one-way ANOVA then separate doses were compared to controls using a Dunnett's test.
Food consumption data were evaluated by Bartlett's test for equality of variances. Exploratory data analysis was performed by a parametric analysis of variance (ANOVA) and if significant at alpha = 0.05, followed by Dunnett's test, alpha = 0.05. The experiment-wise alpha level was reported.
Descriptive statistics only were reported for body weight gains, RBC indices and differential WBC counts. Statistical outliers were identified by a sequential test, and routinely excluded from food consumption statistics. Other outliers may have been excluded only for documented, scientifically sound reasons. DCO incidence scores were qualitatively evaluated.
Because numerous measurements were statistically compared to the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results are consistent with other biological and pathological findings and historical control values.

C. METHODS:

1. **Observations:** Twice daily a cage-side examination was conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Detailed clinical observations (DCO) were conducted on a weekly basis, and included cage-side, hand-held and open-field observations.

2. **Body Weight:** Individual body weights were measured twice during the first week of the study, and once a week thereafter.

3. **Food Consumption and Compound Intake:** Food consumption was measured twice during the first week of the study, and once a week thereafter. From the body weight and food intake data, the actual test material intake per animal (mg/kg bw/day) was calculated.

4. **Ophthalmoscopic Examination:** Eyes of all animals were examined prior to study initiation and prior to termination using indirect ophthalmoscopy, and during necropsy using a moistened glass slide pressed to the cornea.

5. **Hematology & Clinical Chemistry;** Blood samples were taken on the day of necropsy from all animals via the orbital sinus, for hematology and clinical chemistry analysis. Animals were fasted prior to collection, and were anesthetized with methoxyflurane. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

64

a. Hematology:

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
	Blood clotting measurements		Reticulocyte count
✓	(Platelet count)		
	(Thromboplastin time)		
	(Clotting time)		
✓	(Prothrombin time)		

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium		Globulins
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (ALT/also SGPT)		
✓	Serum aspartate amino-transferase (AST/also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Ornithine decarboxylase		

6. Urinalysis: Urine samples were collected from all nonfasted animals during the week prior to necropsy by manual compression of the urinary bladder. The CHECKED (✓) parameters were examined.

✓	Appearance	✓	Glucose
	Volume	✓	Ketones
✓	Specific gravity	✓	Bilirubin
✓	pH	✓	Blood
	Sediment (microscopic)		Nitrate
✓	Protein	✓	Urobilinogen

7. Sacrifice and Pathology: At study termination, animals were anesthetized by inhalation of methoxyfluorane, sacrificed by decapitation, and then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Tongue and oral tissues	✓	Aorta	✓	Brain
				✓	

✓	Salivary glands	✓	Heart	✓	Periph.nerve
✓	Esophagus	✓	Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)
✓	Jejunum	✓	Thymus		**GLANDULAR**
✓	Ileum			✓	Adrenal gland
✓	Cecum		**UROGENITAL**	✓	Lacrimal/Harderian glands
✓	Colon	✓	Kidneys	✓	Mammary gland
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
✓✓	Liver	✓	Testes	✓	Thyroids
	Gall bladder	✓	Epididymides	✓	Auditory sebaceous glands
✓	Pancreas	✓	Prostate	✓	Coagulating glands
	RESPIRATORY	✓	Seminal vesicle		**OTHER**
✓	Trachea	✓	Ovaries and oviducts	✓	Bone
✓	Lung	✓	Uterus	✓	Skeletal muscle
✓	Nasal tissues	✓	Cervix	✓	Skin
	Pharynx	✓	Vagina	✓	All gross lesions and masses
✓	Larynx				

The (✓) tissues were examined from all animals in the control and 1000 mg/kg bw/day groups. In addition, relevant gross lesions were examined for all animals in the 10, 100 and 500 mg/kg bw/day groups.

II. RESULTS

A. Observations

1. Mortality: All animals survived the duration of the study period.

2. Clinical Observations: There were no overt, clinical signs of treatment-related toxicity.

B. Body Weight and Weight Gain: There was no treatment-related effect on body weight or body weight gain.

C. Food Consumption and Compound Intake:

1. Food Consumption: There were no treatment-related findings.

2. Compound Consumption: Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

v8

3. **Food Efficiency**: Not conducted.

D. Ophthalmoscopic Examination: There were no treatment-related findings.

E. Blood Analyses:

1. **Hematology**: Refer to Table 2. The only finding was increased prothrombin time for males in the 1000 mg/kg bw/day group. The study authors did not consider this finding to be toxicologically significant since it fell within the historical control range of mean values. However, the concurrent control value was lower than the historical control range, and the value for the 1000 mg/kg bw/day group fell at the high end of the historical control range. Hence, the PMRA reviewer considers that the slightly higher value noted in the 1000 mg/kg bw/day group could reflect a marginal, treatment-related effect, but concurs that it is not toxicologically significant.

Table 2 - Prothrombin Time (PT) for Males[a]

	Dose, mg/kg bw/day					
	0	10	100	500	1000	Historical control data[b]
PT (sec)	13.1±0.8	13.2±0.9	13.3±0.6	14.1±1.4	15.3±0.6*	14.6-15.6

[a] Data obtained from page 126 in the study report; n=05.
[b] Historical control group mean range from six 4-week dietary studies conducted since 1998; individual range of values were not provided.
* Statistically significantly different from control, $p < 0.05$.

2. **Clinical Chemistry**: There were no treatment-related findings.

F. Urinalysis: There were no treatment-related findings.

G. Sacrifice and Pathology:

1. **Organ Weight**: There were no treatment-related findings.

2. **Gross Pathology**: Refer to Table 3. The only finding considered to be related to treatment was increased size of the cecum, observed in the 500 and 1000 mg/kg bw/day groups, both sexes. In the absence of any correlated histopathological changes, this was not considered to be an adverse effect, reflective of physiological changes in the digestive tract following ingestion of XDE-750.

Table 3 - Selected Gross Pathological Findings in the Cecum[a]

	Dose, mg/kg bw/day				
	0	10	100	500	1000
Increased size					
Males	0	0	0	32	55
Females					

[a] Data obtained from pages 78 and 79 in the study report; n=5.

3. **Microscopic Pathology**: There were no treatment-related findings.

III. DISCUSSION

A. Investigators' Conclusions: "There were no treatment-related effects in body weights, feed consumption, ophthalmologic and clinical observations, organ weights or clinical pathology parameters. The only treatment-related gross observation was increased size of the cecum noted in 3 males and 2 females given 500 mg/kg/day, and 5 males and 5 females given 1000 mg/kg/day. There were no histologic changes associated with the cecal alteration. Therefore, the increased size of the cecum was interpreted to be a non-adverse effect, reflective of physiological changes to the digestive tract following ingestion of XR-750. There were no treatment-related histopathologic observations.
Based on the increased size of the cecum noted in rats given 500 mg/kg/day or 1000 mg/kg/day, the no-observed-effect level (NOEL) for Fischer 344 rats of either sex following 4-weeks of dietary exposure to XR-750 was 100 mg/kg/day. The no-observed-adverse-effect level was 1000 mg/kg/day for both sexes."

B. Reviewer Comments: Male and female Fischer 344 rats were fed test diets containing technical XDE-750, purity 95.4%, at dose levels of 0, 10, 100, 500 or 1000 mg/kg bw/day (equal to 0, 11.4, 113.7, 561.1 or 1125.6 mg/kg bw/day for males, and 0, 12.2, 110.2, 550.5 or 1093.7 mg/kg bw/day for females) for a period of 28 days, 5 rats per sex per group. There were no treatment-related effects on mortality, body weights, food consumption, ophthalmologic and clinical observations, organ weights, clinical chemistry, urinalysis or histopathology.
Slightly higher prothrombin time for males in the 1000 mg/kg bw/day group were considered to possibly reflect a marginal, treatment-related effect, but were not considered adverse since the value fell at the high end of the historical control range of values. The only other finding was increased size of the cecum noted in the 500 and 1000 mg/kg/day groups, both sexes. Since there were no associated histolopathological changes, this was not considered to be an adverse effect, but rather reflective of physiological changes to the digestive tract following ingestion of XR-750.
Based on the results of this study, the LOAEL could not be determined since there were no adverse, treatment-related findings.The NOAEL is 1000 mg/kg bw/day (equal to 1125.6/1093.7 mg/kg bw/day).

C. Study Deficiencies: No scientific deficiencies were noted in the study.

ND

Aminopyralid Liquid Concentrate / DOW ~ PROTECTED ~ aminopyralid [AMD] / PMRA Sub. No. 2004-0790

Rat 90-day dietary toxicity / 1
DACO 4.3.1 / OECD IIA 5.3.2

PMRA Primary Reviewer: Steve Wong, Ph.D.
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division

Signature:

Date Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)

Signature:

Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: 90-Day dietary toxicity - rat; OPPTS 870.3100; OECD 408.

PC CODE: 005100
005209

DP BARCODE: D305670

TEST MATERIAL (purity):

GF-871 (amino-3,6-dichloro-2-propanol: 1,1',1'-nitrilotris-) (aqueous formulation consisting of 41.3% XDE-750 TIPA salt as the active ingredient)

SYNONYMS: XDE-750 triisopropanolammonium (TIPA), X677349

CITATION: KE Stebbins and MD Dryzga, February 25, 2004. GF-871: 90-Day dietary toxicity study in Fischer 344 rats. Toxicology and Environmental Research & Consulting, Midland, MI. Study ID 031140. Unpublished

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

In a 90-day dietary toxicity study (MRID 46235622), GF-871 (containing 41.3 % XDE-750) was administered in the diet to groups of Fischer 344 rats, 10/sex/group, at targeted doses of 0, 465, 1211, or 2421 mg/kg bw/d or 0, 192, 500, or 1000 mg/kg bw/d in terms of XDE-750 TIPA. The acid equivalent (ae) doses are 0,100, 260, and 520 mg ae/kg bw/d. All rats were observed daily for mortality and clinical signs of toxicity. Ophthalmologic examination was conducted prior to dosing and before study termination. Body weight of individual animals was recorded pre-dosing, and then at weekly intervals. Food consumption was recorded weekly. At termination sacrifice, hematology (including prothrombin time), clinical chemistry, urinalysis, selected organ weights, gross and histopathologic examinations were carried out.

There were no treatment-related effects in body weight, feed consumption, ophthalmologic and clinical observations, hematologic and clinical chemistry parameters. Rats, both sexes, given 1211 or 2421 mg/kg bw/d had treatment-related statistically identified increases in absolute and relative full cecal weights. When the ceca were emptied of their contents, the absolute and relative weights of the empty ceca were statistically identified as increased for males given 2421 mg/kg bw/d. The increases in cecal weights were unaccompanied by histopathological changes and were considered to be a non-adverse osmotic effect. The only other treatment-related alterations, which were considered secondary to increased resorption of colonic water with compensatory renal excretion, were minimal increases in urine volume for high-dose males and females, and a minimal decrease in urine specific gravity for high-dose females. The no-observed-adverse effect level (NOAEL) was 2421 mg/kg bw/d or 1000 mg/kg bw/d XDE-750 TIPA. The acid equivalent dose is 520 mg ae/kg bw/d.

The LOAEL is not determined.

This study is acceptable and satisfies the data requirement for a 90-day dietary toxicity study in the rat [DACO 4.3.1; OPPTS 870.3100; OECD 408].

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and data confidentiality statements were provided.
This study was conducted in accordance with the following test guidelines:
1. OECD Guidelines for Testing of Chemicals - Guideline 408, September 21, 1998.
2. US EPA OPPTS 870.4100, August, 1998.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** GF-871
   - **Description:** wine coloured (amber) liquid
   - **Lot/Batch #:** Lot#173-162-1A, Ref# TSN102319
   - **Purity:** contains 41.3 % XDE-750
   - **Compound stability:** The stability of GF-871 was determined in the feed at concentrations of 0.0005, 0.005, and 6% for at least 61 days.

2. Vehicle and/or positive control: LabDiet. Certified Rodent Diet #5002 (PMI Nutrition International)

3. **Test animals:**
   - **Species:** rat
   - **Strain:** Fischer 344
   - **Age/weight at study initiation:** 6-wk old; ♂ = 112.0-138.3 g, ♀ = 85.1-101.3 g
   - **Source:** Charles River Labs Inc, Raleigh, NC, USA
   - **Housing:** individually in stainless steel cage with wire-mesh floors
   - **Diet:** LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO, USA) *ad libitum*
   - **Water:** municipal water *ad libitum*
   - **Environmental conditions:**
     - **Temperature:** 22.0-22.4 °C
     - **Humidity:** 48.0-53.8 %
     - **Air changes:** 12-15 exchanges per hour
     - **Photoperiod:** 12 h dark/ 12h light (0600-1800 h)
   - **Acclimation period:** ≥ 7 days

B. STUDY DESIGN:

1. **In life dates** - Start: October 1, 2003 End: December 30/31, 2003

2. **Animal assignment/dose levels:** Rats were assigned randomly to test groups noted in Table 1.

Table 1: Study design

Targeted GF-871, mg/kg bw/d	GF-871 in diet ppm		GF-871 intake mg/kg bw/d		N		Mortality	
	♂	♀	♂	♀	♂	♀	♂	♀
0	0	0	0	0	10	10	0	0

97

465	4668-9242	4492-7806	483±58	477±32	10	10	0	0
1211	12156-22697	11699-19360	1270±88	1245±80	10	10	0	0
2421	24301-45010	23389-36988	2556±199	2508±173	10	10	0	0
Data taken from Tables 16, pp 64-65								

3. **Dose Selection**:

The dose levels selected were based on results from the XDE-750 90-day study. The high-dose was based on the limit dose for the active ingredient (XDE-750 TIPA) in the formulation and was expected to produce increases in cecal weight. The high dose of 1000 mg/kg bw/d XDE-750 TIPA salt was equivalent to 2421 mg/kg bw/d of GF-871. The mid- and low-dose levels were expected to provide dose response data for any treatment-related effects observed in the high-dose group. The mid-dose of 500 mg/kg bw/d XDE-750 TIPA salt was equivalent to 1211 mg/kg bw/d of GF-871. The low-dose was based on the NOEL of 100 mg/kg bw/d of XDE-750 determined in the 90-day study and was equivalent to 192 mg/kg bw/d of XDE-750 TIPA salt or 465 mg/kg bw/d of GF-871.

4. **Diet preparation and analysis:**

The initial concentrated diets were prepared by mixing the formulation in acetone and dispersing the mixture into ground feed. Successive diets were prepared by serially diluting test material-feed mixtures with ground feed. Diets were prepared weekly based upon the most recent body weight and feed consumption data. Initial concentrations of test material in the diet were calculated from historical body weights and feed consumption data. Control animals received control feed in which equivalent amounts of water representative of the amount of water in the GF-871 formulation and equivalent amounts of acetone were added as in the male high dose. Control feed and the high dose diets (prior to serial diluting) were mixed and left overnight in a vented area before feeding to animals. Dose confirmation analyses of all dose levels and control, were determined pre-exposure, near the middle and end of the study. The homogeneity of the low-dose female and the high-dose male test diets were determined concurrent with dose confirmation. The method used for analyzing the test material in the diet was a solvent extraction method followed by analysis using liquid chromatography-mass spectrometry (LCMS) with internal and external standards. Prior to the start of the study, stability of GF-871 was determined in the feed at concentrations of 0.0005, 0.005, and 6% for 11 days and was further determined during the study out to 61 days.

Results -

Stability analysis:
Stability of GF-871 in rodent diet was initiated prior to the start of the study at concentrations of 0, 0.05, 0.5 and 6.0%. GF-871 was determined to be stable in the rodent diet at all tested concentrations for at least 61 days. The concentrations after 61 days were 100-117% of initial concentrations.

Homogeneity and concentration analyses:
The homogeneity of GF-871 in rodent diet was determined on three separate mixing batches (pre-exposure, near the middle and end of the study) for the 465 mg/kg bw/d female and 2421 mg/kg bw/d male test diets. The diets were homogeneous with relative standard deviations for all diets sampled between 2.53 and 5.71%.

Dose confirmation analysis of all dose levels and control were determined pre-exposure, near the middle and end of the study and were found to be acceptable. LC/MS analysis indicated 85.3-113% of the target concentration of GF-871 for each individual sample. The mean concentration for each dose

level for the three analytical time points ranged from 94.3 to 103% of the targeted concentration. No GF-871 was found in the control diet.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual concentrations was acceptable.

5.. **Statistics** -

Means and standard deviations were calculated for all continuous data. Body weights, food intake, organ weights, urine volume, urine specific gravity, clinical chemistry, coagulation, and appropriate hematological data were evaluated by Barlett's test for equality of variances. Based on the outcome of the Barlett's test, exploratory data analyses were performed by a parametric or non-parametric analysis of variance (ANOVA). If the ANOVA was significant at $\alpha = 0.05$, it was followed respectively by Dunnett's test or the Wilcoxin Rank-Sum test. Detailed clinical observation (DCO) incidence scores were statistically analyzed by a z-test of proportions comparing each treatment group to the control group. Descriptive statistics were reported fo body weight gains, feed efficiency, RBC indices, and differential WBC counts. Statistical outliers were identified by a sequential test ($\alpha = 0.02$), but routinely excluded only from feed consumption calculations. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal α levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

C. **METHODS**:

Prior to the start of dosing, the rats were evaluated to determine the general health status and acceptability. The rats were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. The rats were fed diets containing GF-871 at target levels of 0, 465, 1211, or 2421 mg/kg bw/d

1. **Observations**:

Animals were inspected at least once daily for signs of toxicity, mortality, and moribundity. Detailed clinical observations (DCO) were conducted at pre-exposure and weekly throughout the study. The DCO was conducted on all animals, at approximately the same time each examination day according to an established format. The examination included cage-side, hand-held and open-field observations that were recorded categorically or using explicitly defined scales (ranked). Moribund animals not expected to survive until the next observation period were humanely killed. Any animal found dead was necropsied as soon as was practical.

2. **Body weight**

Body weight of individual animals was measured prior to start of the study, and at weekly intervals.

3. **Food consumption and compound intake:**

Feed consumption data were collected weekly for all animals. Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/d. Actual test material intake (mg/kg bw/d) values were calculated using actual feed concentrations, body weights and feed consumption data.

4. **Ophthalmoscopic examination:**

All eyes were examined by a veterinarian prior to dosing and at the end of the study period.

5. Hematology and clinical chemistry:

Hematology and clinical chemistry were carried out at scheduled sacrifice. Blood samples were collected from the orbital sinus of fasted animals anaesthetized with CO_2.

a. Hematology - The parameters evaluated are x-marked in the following table.

x	hematocrit*	x	platelet count*	x	mean corpuscular Hb (MCH)
x	haemoglobin*	x	leukocyte differential count*	x	mean corpuscular volume (MCV)
x	leukocyte count*	x	mean corpuscular Hb concentration (MCHC)	x	blood clotting prothrombin time*
x	erythrocyte count*				reticulocyte count
* Required for chronic studies based on Subdivision F Guidelines.					

b. Clinical chemistry - The parameters evaluated are x-marked in the following table.

Electrolytes		Other			
x	calcium*	x	albumin*	x	total protein *
x	chloride*	x	creatinine*	x	total bilirubin
	magnesium	x	blood urea nitrogen*	x	glucose*
x	phosphorus*	x	total cholesterol	x	globulins
x	potassium*	x	triglycerides	x	albumin/globulin ratio
x	sodium*				serum protein electrophores
Enzymes					
x	alkaline phosphatase	x	serum alanine amino-transferase (SGPT)*	x	serum aspartate amino-transferase (SGOT)*
				x	γ-glutamyl transferase (GGT)
* Recommended for subchronic rodent studies					

6. Urinalysis

Urinalysis was conducted during the week prior to terminal sacrifice. Urine was collected from non-fasted animals overnight in a metabolism cage (~16 h), as well as by manual compression of the bladder. The following parameters (X) were examined.

x	appearance*	x	glucose*	x	sediment (microscopic)*
x	volume*	x	protein*	x	urobilinogen
x	specific gravity*	x	bilirubin*	x	ketones*
x	pH	x	blood*		nitrate
* Optional for subchronic studies					

7. Sacrifice and pathology

At study termination, fasted animals were sacrificed by CO_2 anaesthesia, then necropsied and assessed by gross pathology. The animals that died intercurrently or were sacrificed in a moribund state were necropsied as soon as possible after death and assessed by gross pathology. The weight of the animals were recorded at sacrifice. The checked (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

n5

	Digestive system		Cardiovascular		Neurologic		Respiratory
	tongue	x	aorta*	xx	brain*+	x	trachea*
x	salivary glands*	xx	heart*+	x	peripheral nerve*	x	lung*
x	esophagus*	x	bone marrow*	x	spinal cord (3 levels)*	x	nose
x	stomach*	x	lymph nodes*	x	pituitary*	x	pharynx
x	duodenum*	xx	spleen*+	x	eyes (optic nerve)*	x	larynx
x	jejunum*	xx	thymus*+				
x	ileum*				Urogenital		Other
xx	cecum*		Glandular	xx	kidneys*+	x	bone (sternum only)*
x	colon*	xx	adrenal gland*+	x	urinary bladder*	x	skeletal muscle*
x	rectum*		lacrimal gland	xx	testes*+	x	skin*
xx	liver*+	x	mammary gland*	xx	epididymides*+	x	gross lesions and masses*
	gall bladder*	x	parathyroid*+	x	prostate	x	cervix
x	pancreas*	x	thyroids*+	x	seminal vesicle	x	coagulation glands
				xx	ovaries*+	x	mediastinal tissues
				xx	uterus*+	x	mesenteric tissues
						x	oral tissues
						x	vagina

* Recommended for subchronic rodent studies
+ Organ weight required for rodent studies

II. RESULTS

A. Observations

1. Clinical signs of toxicity -

There were no statistically significant or treatment related changes in ranked DCO parameters. All clinical observations were considered to be unrelated to treatment.

2. Mortality - There were no deaths.

B. Body weight -

There were no statistically significant or treatment-related effects in body weights or body-weight gains for males or females following 90-days of dietary administration of GF-871.

C. Food consumption and compound intake

1. Food consumption -

Feed consumption of high-dose males was marginally higher than controls from week 4 to study termination. Feed consumption for males and females from the remaining treatment groups was comparable to controls throughout the study.

n4

Table 2: Selected food consumption data of males, g/rat/day, N=10/group

mg/kg bw/d	0	465	1211	2421
days 1-8	14.7±1.0	14.1±0.6	14.6±1.0	14.6±1.1
15-22	17.7±1.0	16.8±0.7	17.2±1.4	17.9±1.5
22-29	17.8±0.7	17.2±0.8	17.7±1.3	18.4±1.3
36-43	17.3±0.9	16.6±0.9	17.0±0.9	17.8±0.9
50-57	17.2±1.0	16.7±0.9	17.3±0.8	17.7±1.0
64-71	16.8±1.2	16.0±0.9	17.3±1.2	17.9±0.7
71-78	17.3±0.6	16.6±0.6	17.8±1.2	**18.4±1.2***
78-85	17.1±0.9	16.6±0.9	17.6±1.1	18.2±1.2
85-90	17.4±1.3	16.4±0.8	18.0±1.3	18.2±1.2
data (mean ± SD) obtained from Table 14, p 62; * $p \leq 0.05$				

2. **Compound consumption (Time-weighed average)** - Data are presented in Table 1.

3. **Food efficiency** - Food efficiency was not assessed.

D. **Ophthalmoscopic examination** - There were no treatment-related findings.

E. **Blood analyses - Hematology and clinical chemistry**

There were no treatment-related changes in hematologic and clinical chemistry parameters.

F. **Urinalysis** -

Table 3: Selected urine parameters

mg/kg bw/d	0	Historical data	465	1211	2421
	♂ (N=10/group)				
volume, mL	5.6±1.1	3.2-5.4	4.9±0.8	6.1±1.3	**7.3±1.7***
specific gravity	1.065±0.008	1.054-1.081	1.069±0.006	1.066±0.004	1.062±0.006
	♀ (N=10/group)				
volume, mL	4.4±1.0	2.9±5.4	5.2±1.5	5.2±1.1	5.6±0.5
specific gravity	1.059±0.007	1.048-1.075	1.055±0.014	1.052±0.010	1.049±0.007
data (mean ± SD) obtained from Tables 28 and 30, pp 76 and 78; * $p \leq 0.05$ Historical control data were group mean range from 6 90-d dietary studies done since 1999					

High-dose males had a statistically identified higher mean urine volume, relative to controls. High-dose females had a slightly higher mean urine volume and a slightly lower mean urine specific gravity, relative to controls, which were not statistically identified. These alterations were interpreted to be treatment-related.

nn

The urine changes were considered adaptive effects to the high levels of GF-871 in the diet. As noted in the following gross pathology section, the ceca of high-dose rats were enlarged by normal semi-solid ingesta. As the feces were normally formed pellets, it was postulated that there was increased colonic water resorption with compensatory renal excretion of the additional water, which led to the somewhat increased urine volume and decreased specific gravity. The alterations in urine volume and urine specific gravity were unaccompanied by treatment-related histopathological changes and were considered to be non-adverse.

G. Sacrifice and pathology

1. Organ weight -

Males and females given 1211 or 2421 mg/kg bw/d had treatment-related statistically identified increases in absolute and relative full (including contents) cecal weights. When the ceca were emptied of their contents, the absolute and relative weights of the empty ceca were statistically identified as increased for high-dose males. The increases in cecal weights were unaccompanied by treatment-related histopathological changes and were considered to be non-adverse. There were no other treatment-related alterations in organ weights of males and females at any dose level.

Table 4: Cecum weight data

mg/kg bw/d		0	465	1211	2421
		♂ (N=10/group, except a = 9)			
full	g	4.727±0.628a	5.343±0.607	**6.111±0.987***	**8.285±0.930***
	g/100	1.549±0.175a	1.771±0.193	**1.986±0.317***	**2.672±0.316***
empty	g	2.071±0.246	2.143±0.145	2.307±0.208	**2.509±0.355***
	g/100	0.681±0.077	0.711±0.044	0.749±0.062	**0.808±0.103***
		♀ (N=10/group)			
full	g	3.844±0.434	3.979±0.221	**4.323±0.393***	**5.696±0.310***
	g/100	2.274±0.251	2.357±0.113	**2.574±0.196***	**3.440±0.216***
empty	g	1.517±0.229	1.420±0.167	1.558±0.196	1.654±0.154
	g/100	0.894±0.096	0.839±0.069	0.928±0.109	0.999±0.108
data (mean ± SD) obtained from Tables 32 and 33; pp 81 and 83; * $p \leq 0.05$					

2. Gross pathology

The only treatment-related gross observation was an increased size of the cecum of all high-dose males and females. There was no histopathologic correlate to the increased cecal size.

3. Microscopic pathology

There were no treatment-related histopathologic observations. All histopathologic observations were considered to be spontaneous alterations, unassociated with exposure to GF-871.

III. DISCUSSION

A. Investigators' conclusions:

"Subchronic dietary exposure to XDE-750 TIPA given in the GF-871 formulation, produced similar findings at equivalent dosages as those seen in the subchronic study conducted with XDE-750 acid. Enlargement of the cecum was the primary treatment-related effect in rats given 1211 or 2421 mg GF-871/kg/day for 13 weeks. The cecal enlargement was interpreted to be reflective of physiological changes in the cecum following ingestion of GF-871. These alterations probably represent an adaptive process since the increases in cecal weights were shown to be partially reversible in a previous 13-week dietary toxicity study with 4-week recovery of XDE-750 in Fischer 344 rats (Dryzga and Stebbins, 2001).

Cecal enlargement has been observed in several rodent species following exposure to various compounds and food additives. These materials include antibiotics, modified starches, polyols (sorbitol and mannitol), some fibers, and lactose (Bertram, 1996; Newberne et al. 1988). These compounds are poorly absorbed and contribute to increased osmolality of the cecal contents. The mechanism for cecal enlargement has been proposed to be the attraction of fluid into the lumen. Other alterations associated with cecal enlargement in rodents include mucosal hypertrophy and hyperplasia. These morphological changes were interpreted to represent an adaptive process since the changes were shown to be reversible when the diets were returned to normal.

There were no treatment-related effects in body weights, feed consumption, ophthalmologic and clinical observations, hematologic and clinical chemistry parameters. The only other treatment-related alterations, which were considered secondary to increased resorption of colonic water with compensatory renal excretion, were minimal increases in urine volume for males and females given 2421 mg/kg/day, and a minimal decrease in urine specific gravity for females given 2421 mg/kg/day.

The no-observed-effect level (NOEL) for Fischer 344 rats of either sex was the targeted concentration of 465 mg/kg/day. The no-observed-adverse effect level (NOAEL) was the targeted concentration of 2421 mg/kg/day for both sexes."

B. Reviewer comments:

The study was well conducted and reported. The NOAEL for both males and females was 2421 mg/kg bw/d, the highest dose tested. This 90-day dietary toxicity study in the rat is acceptable/guideline and satisfies the guideline requirement for a subchronic toxicity study in rats.

C. **Study deficiencies:** There were no deficiencies.

005100
(8)

Aminopyralid Liquid Concentrate / DOW ~ **PROTECTED** ~ **Rat 90-day dietary toxicity / 1**
aminopyralid [AMD] / PMRA Sub. No. 2004-0790 **DACO 4.3.1 / OECD IIA 5.3.2**

PMRA Primary Reviewer: Steve Wong, Ph.D.
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division

Signature: Steve Wong
Date Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)

Signature: Karlyn Bailey
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: 90-Day dietary toxicity - rat; OPPTS 870.3100; OECD 408.

PC CODE: 005100 005209 **DP BARCODE**: D305670

TEST MATERIAL (purity):

GF-871 (amino-3,6-dichloro-2-propanol: 1,1',1'-nitrilotris-) (aqueous formulation consisting of 41.3% XDE-750 TIPA salt as the active ingredient)

SYNONYMS: XDE-750 triisopropanolammonium (TIPA), X677349

CITATION: KE Stebbins and MD Dryzga, February 25, 2004. GF-871: 90-Day dietary toxicity study in Fischer 344 rats. Toxicology and Environmental Research & Consulting, Midland, MI. Study ID 031140. Unpublished

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

In a 90-day dietary toxicity study (MRID 46235622), GF-871 (containing 41.3 % XDE-750) was administered in the diet to groups of Fischer 344 rats, 10/sex/group, at targeted doses of 0, 465, 1211, or 2421 mg/kg bw/d or 0, 192, 500, or 1000 mg/kg bw/d in terms of XDE-750 TIPA. The acid equivalent (ae) doses are 0,100, 260, and 520 mg ae/kg bw/d. All rats were observed daily for mortality and clinical signs of toxicity. Ophthalmologic examination was conducted prior to dosing and before study termination. Body weight of individual animals was recorded pre-dosing, and then at weekly intervals. Food consumption was recorded weekly. At termination sacrifice, hematology (including prothrombin time), clinical chemistry, urinalysis, selected organ weights, gross and histopathologic examinations were carried out.

There were no treatment-related effects in body weight, feed consumption, ophthalmologic and clinical observations, hematologic and clinical chemistry parameters. Rats, both sexes, given 1211 or 2421 mg/kg bw/d had treatment-related statistically identified increases in absolute and relative full cecal weights. When the ceca were emptied of their contents, the absolute and relative weights of the empty ceca were statistically identified as increased for males given 2421 mg/kg bw/d. The increases in cecal weights were unaccompanied by histopathological changes and were considered to be a non-adverse osmotic effect. The only other treatment-related alterations, which were considered secondary to increased resorption of colonic water with compensatory renal excretion, were minimal increases in urine volume for high-dose males and females, and a minimal decrease in urine specific gravity for high-dose females. The no-observed-adverse effect level (NOAEL) was 2421 mg/kg bw/d or 1000 mg/kg bw/d XDE-750 TIPA. The acid equivalent dose is 520 mg ae/kg bw/d.

The LOAEL is not determined.

This study is acceptable and satisfies the data requirement for a 90-day dietary toxicity study in the rat [DACO 4.3.1; OPPTS 870.3100; OECD 408].

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and data confidentiality statements were provided.
This study was conducted in accordance with the following test guidelines:
1. OECD Guidelines for Testing of Chemicals - Guideline 408, September 21, 1998.
2. US EPA OPPTS 870.4100, August, 1998.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** GF-871
   - **Description:** wine coloured (amber) liquid
   - **Lot/Batch #:** Lot#173-162-1A, Ref# TSN102319
   - **Purity:** contains 41.3 % XDE-750
   - **Compound stability:** The stability of GF-871 was determined in the feed at concentrations of 0.0005, 0.005, and 6% for at least 61 days.

2. Vehicle and/or positive control: LabDiet. Certified Rodent Diet #5002 (PMI Nutrition International)

3. **Test animals:**
   - **Species:** rat
   - **Strain:** Fischer 344
   - **Age/weight at study initiation:** 6-wk old; ♂ = 112.0-138.3 g, ♀ = 85.1-101.3 g
   - **Source:** Charles River Labs Inc, Raleigh, NC, USA
   - **Housing:** individually in stainless steel cage with wire-mesh floors
   - **Diet:** LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO, USA) *ad libitum*
   - **Water:** municipal water *ad libitum*
   - **Environmental conditions:**
     - **Temperature:** 22.0-22.4 °C
     - **Humidity:** 48.0-53.8 %
     - **Air changes:** 12-15 exchanges per hour
     - **Photoperiod:** 12 h dark/ 12h light (0600-1800 h)
   - **Acclimation period:** ≥7 days

B. STUDY DESIGN:

1. In life dates - Start: October 1, 2003 End: December 30/31, 2003

2. Animal assignment/dose levels: Rats were assigned randomly to test groups noted in Table 1.

Table 1: Study design

Targeted GF-871, mg/kg bw/d	GF-871 in diet ppm		GF-871 intake mg/kg bw/d		N		Mortality	
	♂	♀	♂	♀	♂	♀	♂	♀
0	0	0	0	0	10	10	0	0

81

465	4668-9242	4492-7806	483±58	477±32	10	10	0	0
1211	12156-22697	11699-19360	1270±88	1245±80	10	10	0	0
2421	24301-45010	23389-36988	2556±199	2508±173	10	10	0	0
Data taken from Tables 16, pp 64-65								

3. **Dose Selection**:

The dose levels selected were based on results from the XDE-750 90-day study. The high-dose was based on the limit dose for the active ingredient (XDE-750 TIPA) in the formulation and was expected to produce increases in cecal weight. The high dose of 1000 mg/kg bw/d XDE-750 TIPA salt was equivalent to 2421 mg/kg bw/d of GF-871. The mid- and low-dose levels were expected to provide dose response data for any treatment-related effects observed in the high-dose group. The mid-dose of 500 mg/kg bw/d XDE-750 TIPA salt was equivalent to 1211 mg/kg bw/d of GF-871. The low-dose was based on the NOEL of 100 mg/kg bw/d of XDE-750 determined in the 90-day study and was equivalent to 192 mg/kg bw/d of XDE-750 TIPA salt or 465 mg/kg bw/d of GF-871.

4. **Diet preparation and analysis:**

The initial concentrated diets were prepared by mixing the formulation in acetone and dispersing the mixture into ground feed. Successive diets were prepared by serially diluting test material-feed mixtures with ground feed. Diets were prepared weekly based upon the most recent body weight and feed consumption data. Initial concentrations of test material in the diet were calculated from historical body weights and feed consumption data. Control animals received control feed in which equivalent amounts of water representative of the amount of water in the GF-871 formulation and equivalent amounts of acetone were added as in the male high dose. Control feed and the high dose diets (prior to serial diluting) were mixed and left overnight in a vented area before feeding to animals. Dose confirmation analyses of all dose levels and control, were determined pre-exposure, near the middle and end of the study. The homogeneity of the low-dose female and the high-dose male test diets were determined concurrent with dose confirmation. The method used for analyzing the test material in the diet was a solvent extraction method followed by analysis using liquid chromatography-mass spectrometry (LCMS) with internal and external standards. Prior to the start of the study, stability of GF-871 was determined in the feed at concentrations of 0.0005, 0.005, and 6% for 11 days and was further determined during the study out to 61 days.

Results -

Stability analysis:
Stability of GF-871 in rodent diet was initiated prior to the start of the study at concentrations of 0, 0.05, 0.5 and 6.0%. GF-871 was determined to be stable in the rodent diet at all tested concentrations for at least 61 days. The concentrations after 61 days were 100-117% of initial concentrations.

Homogeneity and concentration analyses:
The homogeneity of GF-871 in rodent diet was determined on three separate mixing batches (pre-exposure, near the middle and end of the study) for the 465 mg/kg bw/d female and 2421 mg/kg bw/d male test diets. The diets were homogeneous with relative standard deviations for all diets sampled between 2.53 and 5.71%.

Dose confirmation analysis of all dose levels and control were determined pre-exposure, near the middle and end of the study and were found to be acceptable. LC/MS analysis indicated 85.3-113% of the target concentration of GF-871 for each individual sample. The mean concentration for each dose

level for the three analytical time points ranged from 94.3 to 103% of the targeted concentration. No GF-871 was found in the control diet.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual concentrations was acceptable.

5.. **Statistics** -

Means and standard deviations were calculated for all continuous data. Body weights, food intake, organ weights, urine volume, urine specific gravity, clinical chemistry, coagulation, and appropriate hematological data were evaluated by Barlett's test for equality of variances. Based on the outcome of the Barlett's test, exploratory data analyses were performed by a parametric or non-parametric analysis of variance (ANOVA). If the ANOVA was significant at $\alpha = 0.05$, it was followed respectively by Dunnett's test or the Wilcoxin Rank-Sum test. Detailed clinical observation (DCO) incidence scores were statistically analyzed by a z-test of proportions comparing each treatment group to the control group. Descriptive statistics were reported fo body weight gains, feed efficiency, RBC indices, and differential WBC counts. Statistical outliers were identified by a sequential test ($\alpha = 0.02$), but routinely excluded only from feed consumption calculations. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal α levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

C. **METHODS**:

Prior to the start of dosing, the rats were evaluated to determine the general health status and acceptability. The rats were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. The rats were fed diets containing GF-871 at target levels of 0, 465, 1211, or 2421 mg/kg bw/d

1. **Observations**:

Animals were inspected at least once daily for signs of toxicity, mortality, and moribundity. Detailed clinical observations (DCO) were conducted at pre-exposure and weekly throughout the study. The DCO was conducted on all animals, at approximately the same time each examination day according to an established format. The examination included cage-side, hand-held and open-field observations that were recorded categorically or using explicitly defined scales (ranked). Moribund animals not expected to survive until the next observation period were humanely killed. Any animal found dead was necropsied as soon as was practical.

2. **Body weight**

Body weight of individual animals was measured prior to start of the study, and at weekly intervals.

3. **Food consumption and compound intake:**

Feed consumption data were collected weekly for all animals. Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/d. Actual test material intake (mg/kg bw/d) values were calculated using actual feed concentrations, body weights and feed consumption data.

4. **Ophthalmoscopic examination:**

All eyes were examined by a veterinarian prior to dosing and at the end of the study period.

5. Hematology and clinical chemistry:

Hematology and clinical chemistry were carried out at scheduled sacrifice. Blood samples were collected from the orbital sinus of fasted animals anaesthetized with CO_2.

a. Hematology - The parameters evaluated are x-marked in the following table.

x	hematocrit*	x	platelet count*	x	mean corpuscular Hb (MCH)
x	haemoglobin*	x	leukocyte differential count*	x	mean corpuscular volume (MCV)
x	leukocyte count*	x	mean corpuscular Hb concentration (MCHC)	x	blood clotting prothrombin time*
x	erythrocyte count*				reticulocyte count
* Required for chronic studies based on Subdivision F Guidelines.					

b. Clinical chemistry - The parameters evaluated are x-marked in the following table.

Electrolytes		**Other**			
x	*calcium**	x	*albumin**	x	total protein *
x	chloride*	x	creatinine*	x	total bilirubin
	magnesium	x	blood urea nitrogen*	x	glucose*
x	phosphorus*	x	total cholesterol	x	globulins
x	potassium*	x	triglycerides	x	albumin/globulin ratio
x	sodium*				serum protein electrophores
Enzymes					
x	alkaline phosphatase	x	serum alanine amino-transferase (SGPT)*	x	serum aspartate amino-transferase (SGOT)*
				x	γ-glutamyl transferase (GGT)
* Recommended for subchronic rodent studies					

6. Urinalysis

Urinalysis was conducted during the week prior to terminal sacrifice. Urine was collected from non-fasted animals overnight in a metabolism cage (~16 h), as well as by manual compression of the bladder. The following parameters (X) were examined.

x	appearance*	x	glucose*	x	sediment (microscopic)*
x	volume*	x	protein*	x	urobilinogen
x	specific gravity*	x	bilirubin*	x	ketones*
x	pH	x	blood*		nitrate
* Optional for subchronic studies					

7. Sacrifice and pathology

At study termination, fasted animals were sacrificed by CO_2 anaesthesia, then necropsied and assessed by gross pathology. The animals that died intercurrently or were sacrificed in a moribund state were necropsied as soon as possible after death and assessed by gross pathology. The weight of the animals were recorded at sacrifice. The checked (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	Digestive system		Cardiovascular		Neurologic		Respiratory
	tongue	x	aorta*	xx	brain*+	x	trachea*
x	salivary glands*	xx	heart*+	x	peripheral nerve*	x	lung*
x	esophagus*	x	bone marrow*	x	spinal cord (3 levels)*	x	nose
x	stomach*	x	lymph nodes*	x	pituitary*	x	pharynx
x	duodenum*	xx	spleen*+	x	eyes (optic nerve)*	x	larynx
x	jejunum*	xx	thymus*+				
x	ileum*				**Urogenital**		**Other**
xx	cecum*		**Glandular**	xx	kidneys*+	x	bone (sternum only)*
x	colon*	xx	adrenal gland*+	x	urinary bladder*	x	skeletal muscle*
x	rectum*		lacrimal gland	xx	testes*+	x	skin*
xx	liver*+	x	mammary gland*	xx	epididymides*+	x	gross lesions and masses*
	gall bladder*	x	parathyroid*+†	x	prostate	x	cervix
x	pancreas*	x	thyroids*+†	x	seminal vesicle	x	coagulation glands
				xx	ovaries*+	x	mediastinal tissues
				xx	uterus*+	x	mesenteric tissues
						x	oral tissues
						x	vagina

* Recommended for subchronic rodent studies
† Organ weight required for rodent studies

II. RESULTS

A. Observations

1. Clinical signs of toxicity -

There were no statistically significant or treatment related changes in ranked DCO parameters. All clinical observations were considered to be unrelated to treatment.

2. Mortality - There were no deaths.

B. Body weight -

There were no statistically significant or treatment-related effects in body weights or body-weight gains for males or females following 90-days of dietary administration of GF-871.

C. Food consumption and compound intake

1. Food consumption -

Feed consumption of high-dose males was marginally higher than controls from week 4 to study termination. Feed consumption for males and females from the remaining treatment groups was comparable to controls throughout the study.

Table 2: Selected food consumption data of males, g/rat/day, N=10/group

mg/kg bw/d	0	465	1211	2421
days 1-8	14.7±1.0	14.1±0.6	14.6±1.0	14.6±1.1
15-22	17.7±1.0	16.8±0.7	17.2±1.4	17.9±1.5
22-29	17.8±0.7	17.2±0.8	17.7±1.3	18.4±1.3
36-43	17.3±0.9	16.6±0.9	17.0±0.9	17.8±0.9
50-57	17.2±1.0	16.7±0.9	17.3±0.8	17.7±1.0
64-71	16.8±1.2	16.0±0.9	17.3±1.2	17.9±0.7
71-78	17.3±0.6	16.6±0.6	17.8±1.2	**18.4±1.2***
78-85	17.1±0.9	16.6±0.9	17.6±1.1	18.2±1.2
85-90	17.4±1.3	16.4±0.8	18.0±1.3	18.2±1.2
data (mean ± SD) obtained from Table 14, p 62; * p ≤0.05				

2. **Compound consumption (Time-weighed average)** - Data are presented in Table 1.

3. **Food efficiency** - Food efficiency was not assessed.

D. **Ophthalmoscopic examination** - There were no treatment-related findings.

E. **Blood analyses - Hematology and clinical chemistry**

There were no treatment-related changes in hematologic and clinical chemistry parameters.

F. **Urinalysis** -

Table 3: Selected urine parameters

mg/kg bw/d	0	Historical data	465	1211	2421
	♂ (N=10/group)				
volume, mL	5.6±1.1	3.2-5.4	4.9±0.8	6.1±1.3	**7.3±1.7***
specific gravity	1.065±0.008	1.054-1.081	1.069±0.006	1.066±0.004	1.062±0.006
	♀ (N=10/group)				
volume, mL	4.4±1.0	2.9±5.4	5.2±1.5	5.2±1.1	5.6±0.5
specific gravity	1.059±0.007	1.048-1.075	1.055±0.014	1.052±0.010	1.049±0.007
data (mean ± SD) obtained from Tables 28 and 30, pp 76 and 78; * p ≤0.05 Historical control data were group mean range from 6 90-d dietary studies done since 1999					

High-dose males had a statistically identified higher mean urine volume, relative to controls. High-dose females had a slightly higher mean urine volume and a slightly lower mean urine specific gravity, relative to controls, which were not statistically identified. These alterations were interpreted to be treatment-related.

86

The urine changes were considered adaptive effects to the high levels of GF-871 in the diet. As noted in the following gross pathology section, the ceca of high-dose rats were enlarged by normal semi-solid ingesta. As the feces were normally formed pellets, it was postulated that there was increased colonic water resorption with compensatory renal excretion of the additional water, which led to the somewhat increased urine volume and decreased specific gravity. The alterations in urine volume and urine specific gravity were unaccompanied by treatment-related histopathological changes and were considered to be non-adverse.

G. Sacrifice and pathology

1. Organ weight -

Males and females given 1211 or 2421 mg/kg bw/d had treatment-related statistically identified increases in absolute and relative full (including contents) cecal weights. When the ceca were emptied of their contents, the absolute and relative weights of the empty ceca were statistically identified as increased for high-dose males. The increases in cecal weights were unaccompanied by treatment-related histopathological changes and were considered to be non-adverse. There were no other treatment-related alterations in organ weights of males and females at any dose level.

Table 4: Cecum weight data

mg/kg bw/d		0	465	1211	2421
		♂ (N=10/group, except a = 9)			
full	g	4.727±0.628a	5.343±0.607	**6.111±0.987***	**8.285±0.930***
	g/100	1.549±0.175a	1.771±0.193	**1.986±0.317***	**2.672±0.316***
empty	g	2.071±0.246	2.143±0.145	2.307±0.208	**2.509±0.355***
	g/100	0.681±0.077	0.711±0.044	0.749±0.062	**0.808±0.103***
		♀ (N=10/group)			
full	g	3.844±0.434	3.979±0.221	**4.323±0.393***	**5.696±0.310***
	g/100	2.274±0.251	2.357±0.113	**2.574±0.196***	**3.440±0.216***
empty	g	1.517±0.229	1.420±0.167	1.558±0.196	1.654±0.154
	g/100	0.894±0.096	0.839±0.069	0.928±0.109	0.999±0.108
data (mean ± SD) obtained from Tables 32 and 33; pp 81 and 83; * p ≤0.05					

2. Gross pathology

The only treatment-related gross observation was an increased size of the cecum of all high-dose males and females. There was no histopathologic correlate to the increased cecal size.

3. Microscopic pathology

There were no treatment-related histopathologic observations. All histopathologic observations were considered to be spontaneous alterations, unassociated with exposure to GF-871.

III. DISCUSSION

A. Investigators' conclusions:

"Subchronic dietary exposure to XDE-750 TIPA given in the GF-871 formulation, produced similar findings at equivalent dosages as those seen in the subchronic study conducted with XDE-750 acid. Enlargement of the cecum was the primary treatment-related effect in rats given 1211 or 2421 mg GF-871/kg/day for 13 weeks. The cecal enlargement was interpreted to be reflective of physiological changes in the cecum following ingestion of GF-871. These alterations probably represent an adaptive process since the increases in cecal weights were shown to be partially reversible in a previous 13-week dietary toxicity study with 4-week recovery of XDE-750 in Fischer 344 rats (Dryzga and Stebbins, 2001).

Cecal enlargement has been observed in several rodent species following exposure to various compounds and food additives. These materials include antibiotics, modified starches, polyols (sorbitol and mannitol), some fibers, and lactose (Bertram, 1996; Newberne et al. 1988). These compounds are poorly absorbed and contribute to increased osmolality of the cecal contents. The mechanism for cecal enlargement has been proposed to be the attraction of fluid into the lumen. Other alterations associated with cecal enlargement in rodents include mucosal hypertrophy and hyperplasia. These morphological changes were interpreted to represent an adaptive process since the changes were shown to be reversible when the diets were returned to normal.

There were no treatment-related effects in body weights, feed consumption, ophthalmologic and clinical observations, hematologic and clinical chemistry parameters. The only other treatment-related alterations, which were considered secondary to increased resorption of colonic water with compensatory renal excretion, were minimal increases in urine volume for males and females given 2421 mg/kg/day, and a minimal decrease in urine specific gravity for females given 2421 mg/kg/day.

The no-observed-effect level (NOEL) for Fischer 344 rats of either sex was the targeted concentration of 465 mg/kg/day. The no-observed-adverse effect level (NOAEL) was the targeted concentration of 2421 mg/kg/day for both sexes."

B. Reviewer comments:

The study was well conducted and reported. The NOAEL for both males and females was 2421 mg/kg bw/d, the highest dose tested. This 90-day dietary toxicity study in the rat is acceptable/guideline and satisfies the guideline requirement for a subchronic toxicity study in rats.

C. Study deficiencies: There were no deficiencies.

88 99

005100
(9)

PMRA Sub. No. 2004-0789/DOW AMINOPYRALID/AMD — ~ PROTECTED ~ — Repeat-Dose Oral Toxicity / 1 DACO 4.3.3 / OECD IIA 5.3.1

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** [signature]
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:** [signature]
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Repeat-Dose Dietary Toxicity Study in Dogs; OECD 407.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 95.4% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Stebbins, K.E., et al (2000) XDE-750: 4-Week Dietary Toxicity Study in Beagle Dogs. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 001030, October 4, 2000. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a repeat-dose feeding toxicity study (MRID 46235620), XDE-750 Technical, purity 95.4%, was administered to 2 Beagles per sex per group in the diet at concentrations of 0, 0.15%, 0.45% or 1.5% or 0, 1500, 4500, and 15000 ppm (equal to 0, 62, 93 or 543 mg/kg bw/day for males, and 0, 62, 177 or 556 mg/kg bw/day for females) for a period of 4 weeks. There were no treatment-related effects on body weights, ophthalmologic and clinical observations, clinical pathology parameters, organ weights, gross and histopathological examinations. Food intake was lower in the 3.0% group, both sexes, but was not considered adverse in the absence of any treatment-related effect on body weight gain. The only other findings were decreased RBC count, Hgb and HCT, noted for females at all dose levels. However, these effects were marginal and fell close to historical control values. In addition, there were no corresponding histopathological findings, and so these were not considered to be adverse effects. **The LOAEL could not be determined since there were no adverse, treatment-related effects. The NOAEL is 1.5% or 15,000 ppm (equal to 543 mg/kg bw/day for males and 556 mg/kg bw/day for females).**

This 28-day feeding toxicity study in the dog is classified as ~~supplementary~~ acceptable/non-guideline. It does not satisfy the guideline requirement for a repeat-dose oral study (OPPTS 870.3150, [§82-1]; OECD 407) in dogs. However, it was a preliminary study conducted to aid in the determination of the dose levels to be used for the 13-week and 52-week oral toxicity studies. The study is considered acceptable for this purpose.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-125; TSN102095
Purity:	95.4% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5007 Certified Canine Lab Diet).

3 Test Animals:

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	5 months of age; Males, 6.751 kg to 7.930 kg; Females, 6.006 kg to 7.333 kg.	
Source:	Marshall Research Laboratory, North Rose, NY.	
Housing:	Individually in pens, ~ 3 feet wide x 5 feet long x 5 feet high with plastic coated grid floors.	
Diet:	Purina Certified Canine Lab Diet # 5007 in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	21-23°C
	Humidity:	46-65%
	Air changes:	12-15/hr
	Photoperiod:	12hrs dark/12 hrs light
Acclimation period:	25 days	

B. STUDY DESIGN:

1. **In Life Dates:** May 29, 2000 to June 27, 2000.

2. **Animal Assignment:** Animals were stratified by body weight and then randomly assigned to the test groups noted in Table 1 using a computer program.

TABLE 1 - Study Design

Test Group	Conc. in Diet (%)	Dose to Animal (mg/kg bw/day)	# Male	# Female
Control	0	37986	2	2
Low	0.15	62/62	2	2
Mid	0.45	193/177	2	2
High	1.5	543/556	2	2

3. **Diet Preparation and Analysis:** Fresh diets were prepared on a weekly basis. The high-dose diet was prepared by mixing test material with control diet to obtain the desired percent of test material in the diet. Mid- and low-dose diets were prepared by serially diluting the high-dose level diet with control diet.

Stability of the test material in diet was determined at the low-and high-dose levels after storage for 0 and 13 days. Homogeneity of mixing was not determined. Actual test material concentration in the diet was determined for all dose levels from test diet prepared just prior to study initiation.

Results - Homogeneity Analysis: Not conducted.

Stability Analysis: The actual concentration of XDE-750 in the 0.15% and 1.5% test diets, expressed as percentage of the nominal concentration, were as follows:

Dose (ppm)		
Storage Interval	**0.15%**	**1.5%**
Day 0	97.3%	100.0%
Day 13	92.0%	93.3%

Concentration Analysis: The actual test material concentration of XDE-750 in the 0.15%, 0.45% and 1.5% test diets prepared prior to study initiation, expressed as percentage of the nominal concentration, were 97%, 100% and 100%, respectively.

The analytical data indicated that the stability and the actual concentration of XDE-750 in the test diets was adequate. The homogeneity of the test material in the diet was not determined.

4. Statistics: Descriptive statistics only (means and standard deviations) were performed on food consumption data, body weights, terminal body weights, organ weights, urine specific gravity, coagulation parameters, hematologic parameters and clinical chemistry parameters.

C. METHODS:

1. Clinical Observations: Twice daily a cage-side examination was conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Detailed clinical observations (DCO) were conducted on a weekly basis, and included careful, hands-on evaluations of the skin, fur, mucous membranes, respiration, nervous system function, swelling, masses and behavior.

2. Body Weight: Individual body weights were measured twice during the first week of the study, and once a week thereafter.

3. Food consumption and compound intake: Food consumption was measured twice during the first week of the study, and once a week thereafter. From the body weight and food intake data, the actual test material intake per animal (mg/kg bw/day) was calculated.

4. Ophthalmoscopic Examination: Eyes of all animals were examined prior to study initiation and during the week prior to necropsy using indirect ophthalmoscopy, and during necropsy using a moistened glass slide pressed to the cornea.

5. Hematology & Clinical Chemistry: Blood samples were taken pre-exposure and on the day of

necropsy from all animals via the jugular vein, for hematology and clinical chemistry analysis. Animals were fasted prior to collection. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

a. Hematology

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
	Platelet count		Reticulocyte count
	Blood clotting measurements		
	Thromboplastin time		
	Clotting time		
✓	Prothrombin time		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium		Globulins
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (ALT/also SGPT)		
✓	Serum aspartate amino-transferase (AST/also SGOT)		
✓	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Ornithine decarboxylase		

6. Urinalysis: Urine samples were collected from all animals during the scheduled necropsy by urinary bladder aspiration. The CHECKED (✓) parameters were examined.

✓	Appearance	✓	Glucose
	Volume	✓	Ketones
✓	Specific gravity	✓	Bilirubin
✓	pH	✓	Blood
	Sediment (microscopic)		Nitrate
✓	Protein	✓	Urobilinogen

7. Sacrifice and Pathology: At study termination, animals were tranquilized by SC administration of

acepromazine, subsequently sacrificed by an IV overdose of sodium pentobarbital and exsanguination, and were then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Oral tissues and tongue		Aorta	✓	Brain
				✓	
✓	Salivary glands	✓	Heart	✓	Periph.nerve
		✓			
✓	Esophagus		Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach		Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)
		✓			
✓	Jejunum	✓	Thymus		GLANDULAR
		✓			
✓	Ileum			✓	Adrenal gland
				✓	
✓	Cecum		UROGENITAL		Lacrimal gland
✓	Colon	✓	Kidneys	✓	Mammary gland
		✓			
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
				✓	
✓✓	Liver	✓	Testes	✓	Thyroids
		✓		✓	
✓	Gall bladder	✓	Epididymides		OTHER
		✓			
✓	Pancreas	✓	Prostate	✓	Bone
	RESPIRATORY		Seminal vesicle	✓	Skeletal muscle
✓	Trachea	✓	Ovaries with oviducts	✓	Skin
		✓			
✓	Lung	✓	Uterus	✓	All gross lesions and masses
		✓			
✓	Nose	✓	Cervix		
✓	Pharynx				
✓	Tonsils				

The (✓) tissues were examined from all animals in the control and 1.5% groups. In addition, relevant gross lesions were examined for all animals in the 0.15% and 0.45% groups.

II. RESULTS

A. Observations

1. **Mortality**: All animals survived the duration of the study period.

2. **Clinical Observations**: There were no overt, clinical signs of treatment-related toxicity.

B. *Body Weight and Body Weight Gain*: There was no treatment-related effect on body weight or body

weight gain.

C. **Food Consumption and Compound Intake**

1. **Food Consumption:** Food intake was lower in the 1.5% group, both sexes. However, in the absence of any treatment-related effects on body weight gain, this was not considered to be an adverse effect. Daily mean food intake values (percent of control group value in brackets) for the 0, 0.15%, 0.45% and 1.5% groups, respectively, were as follows:
i) For males: 0.334 kg, 0.326 kg (97.6%), 0.322 kg (96.4%) and 0.267 kg (79.9%).
ii) For females: 0.267 kg, 0.287 kg (107.5%), 0.257 kg (96.3%) and 0.238 kg (89.1%).

2. **Compound Consumption:** Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

3. **Food Efficiency:** Not conducted.

D. **Ophthalmoscopic Examination:** There were no treatment-related findings.

E. **Blood Analyses**

1. **Hematology:** a) Females: One female in the 0.15% group, and both females in the 0.45% and 1.5% groups had slightly lower RBC, hemoglobin and/or hematocrit values. The study author did not consider these findings to be treatment-related since the values were within or near historical control values for 4-week dietary toxicity dog studies conducted at the same laboratory, and also since there were no corresponding histopathological changes. In addition, the study author stated that there was no dose response. However, since the values did appear to decrease with increasing dose level, and the values at all dose levels were lower than the concurrent control values, the PMRA reviewer considers these findingscould possibly reflect a marginal, treatment-related effect, but in the absence of any corresponding histopathological findings does not consider this to be adverse. The longer term dog studies will aid in determining the significance of these noted effects.
b) Males: There were no treatment-related findings.

TABLE 2 - Selected Hematological Findings for Females[a]

Dose	Animal Number	RBC ($x10^6$ µL)	Hgb (g/dL)	HCT (%)
0%	23932394	7.26 6.87	16.5 15.9	48.7 46.6
0.15%	23952396	6.97 6.12	15.9 14.5	46.0 42.3
0.45%	23972398	7.21 6.33	14.3 14.6	42.5 42.6
1.5%	23992400	6.71 6.03	14.6 14.3	41.6 41.5
Historical Control Values[b]		Range: 6.15 - 7.11	Range: 13.9 - 16.3	Range: 43.4 - 50.6

[a] Data obtained from page 23 in the study report
[b] Historical control data obtained from 4-week hematology data from 8 dietary toxicity studies conducted since 1997.

2. Clinical Chemistry: There were no treatment-related findings.

F. Urinalysis: There were no treatment-related findings.

G. Sacrifice and Pathology:

1. Organ Weight: There were no treatment-related findings.

2. Gross Pathology: There were no treatment-related findings.

3. Microscopic Pathology: There were no treatment-related findings.

III. DISCUSSION

A. Investigators' Conclusions: "Male dogs given 1.5% XDE-750 had slightly lower feed consumption, relative to controls, during weeks 2-4. The feed consumption of males given 0.15% or 0.45%, and all female treated groups, was comparable to controls. There were no treatment-related effects in clinical appearance, ophthalmologic examinations, body weight, clinical chemistry (including prothrombin time), hematology, urinalysis, selected organ weights, gross and histopathologic examinations. Under the conditions of this study, the no-observed-effect level (NOEL) for females was 1.5% (556 mg/kg/day) and 0.45% (193 mg/kg/day) for males, based on slightly lower feed consumption of males at the 1.5% dose level. The No-observed-adverse-effect level (NOAEL) for males was 1.5% (543 mg/kg/day).

B. Reviewer Comments: Male and female beagle dogs were fed test diets containing XDE-750, purity 95.4%, at concentrations of 0, 0.15%, 0.45% or 1.5% (equal to 0, 62, 193 or 543 mg/kg bw/day for males, and 0, 62, 177 or 556 mg/kg bw/day for females) for a period of 4 weeks, 2 dogs per sex per group. There were no treatment-related effects on body weights, ophthalmologic and clinical observations, clinical pathology parameters, organ weights, gross and histopathological examinations. Food intake was lower in the 3.0% group, both sexes, but was not considered adverse in the absence of any treatment-related effect on body weight gain. The only other findings were decreased RBC count, Hgb and HCT, noted for females at all dose levels. However, these effects were marginal and fell close to historical control values. In addition, there were no corresponding histopathological findings, and so these were not considered to be adverse effects.
Based on the results of this study, the LOAEL could not be determined since there were no adverse, treatment-related effects. The NOAEL was 1.5% (equal to 543 mg/kg bw/day for males and 556 mg/kg bw/day for females).

C. Study Deficiencies: No scientific deficiencies were noted in the study.

PMRA Primary Reviewer: Brenda MacDonald, DVM
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature:
Date Aug 31 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)
Signature:
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Study in Dogs; OPPTS 870.3150 (non-rodent); OECD 409.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Stebbins, K.E., et al (2002) XDE-750: 13-Week Dietary Toxicity Study in Beagle Dogs. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 001239R, February 6, 2002. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 46235623), XDE-750 Technical, purity 94.5%, was administered to 4 Beagles per sex per group in the diet at concentrations of 0, 0.15%, 0.75% or 3.0% (equal to 0, 54.5, 282 or 1070 mg/kg bw/day for males, and 0, 52.7, 232 or 929 mg/kg bw/day for females) for a period of 13 weeks, 4 dogs per sex per group. There were no treatment-related effects on body weights, food consumption, ophthalmologic and clinical observations or clinical pathology parameters. Liver weights were increased in the 3.0% group, both sexes. However, in the absence of any corresponding clinical chemistry findings or gross/microscopic changes to the liver, this effect was considered adaptive rather than adverse. Treatment-related histopathological changes to the stomach were observed for all animals in the 3.0% group, manifest as slight diffuse hyperplasia and hypertrophy of the mucosal epithelium. **The LOAEL is 3.0% (equal to 1070 mg/kg bw/day for males and 929 mg/kg bw/day for females), based on stomach histopathology. The NOAEL is 0.75% (equal to 282 mg/kg bw/day for males and 232 mg/kg bw/day for females).**

This subchronic toxicity study in the dog is acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3150; OECD 409) in dogs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-750
   - **Description:** Technical; tan powder.
   - **Lot/Batch #:** F-0031-143; TSN102319
   - **Purity:** 94.5% a.i.
   - **Compound Stability:** Not stated.
   - **CAS #:** CAS# 150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5007 Certified Canine Lab Diet).

3. **Test Animals:**
   - **Species:** Dog
   - **Strain:** Beagle
   - **Age/weight at study initiation:** 5-6 months of age; Males, 6.165 kg to 9.304 kg; Females, 5.994 kg to 7.711 kg.
   - **Source:** Marshall Farms USA, Inc., North Rose, NY.
   - **Housing:** Individually in pens, ~ 1 m wide x 2 m long x 1.5 m high with plastic coated grid floors.
   - **Diet:** Purina Certified Canine Lab Diet # 5007 in meal form, *ad libitum*
   - **Water:** Municiple water, *ad libitum*
   - **Environmental conditions:** **Temperature:** 20 to 25°C; **Humidity:** 49 to 68%; **Air changes:** 12-15/hr; **Photoperiod:** 12 hrs dark/12 hrs light
   - **Acclimation period:** 21 days

B. STUDY DESIGN:

1. **In Life Dates:** February 28, 2001 to June 1, 2001.

2. **Animal Assignment:** Animals were stratified by body weight and then randomly assigned to the test groups noted in Table 1 using a computer program. Littermates were not included in the same treatment groups.

TABLE 1 - Study Design

Test Group	Conc. in Diet (%)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	0/0	4	4
Low	0.15	54.5/52.7	4	4
Mid	0.75	282/232	4	4
High	3.0	1070/929	4	4

Concentrations of test material in the diet were based on the percent in the diets from the 4-week study in Beagles. The high-dose level of 3% represented the limit test dose. The remaining dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose

group and to ensure definition of a no-observed-effect level (NOEL).

3. **Diet Preparation and Analysis:** Fresh diets were prepared at least every two weeks. The high-dose diet was prepared by mixing test material with control diet to obtain the desired percent of test material in the diet. Mid-dose diets were prepared by serially diluting the high-dose level diet with control diet, and the low-dose diets were prepared by diluting the mid-dose level diet with control diet. Stability of the test material in diet was determined at the low-and high-dose levels after storage for 0, 14 and 21 days. Homogeneity of mixing was determined for the low- and high-dose diets prior to study initiation, approximately mid-way through the study and near study termination. Actual test material concentration in the diet was determined for all dose levels from test diets prepared just prior to study initiation, approximately mid-way through the study and near the end of the study.

Results - Homogeneity Analysis: Individual samples of 3 separate batches of the 0.15% test diets ranged from 96.7% to 101.3%, 88.7% to 94.7% and 76.7% to 92.7% of the nominal concentration, respectively.
Individual samples of 3 separate batches of the 3.00% test diets ranged from 98.7% to 103.3%, 99.7% to 106.3% and 99.7% to 102.0% of the nominal concentration, respectively.

Stability Analysis: The actual concentration of XDE-750 in the 0.15% and 3.0% test diets, expressed as percentage of the nominal concentration, were as follows:

	Dose (ppm)	
Storage Interval	**0.15%**	**3.0%**
Day 0	93.3%	105.0%
Day 14	102.0%	112.7%
Day 21	100.7%	116.3%

Concentration Analysis: Individual samples of test diets at all dose levels ranged from 83% to 102% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Means and standard deviations were calculated for all continuous data. All parameters examined statistically were first evaluated by Bartlett's test for equality of variances. If the results from Bartlett's test were significant at alpha = 0.01, then the data for the parameter may have been subjected to a transformation to obtain equality of the variances. The transformations that were examined were the common log, the inverse and the square root. The data were reviewed and an appropriate form of the data was selected. The selected form of the data was then subjected to the appropriate parametric analysis as described below.
In-life body weight, urine volume, urine specific gravity, hematologic parameters (excluding RBC indices and differential WBC) and clinical chemistry parameters were evaluated using a repeated measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex and dose. In the RM-ANOVA, differences between the groups were detected primarily by the time-dose interaction.
The first examination in the RM-ANOVA was the time-sex-dose interaction. If significant at alpha = 0.02, the analysis was repeated separately for each sex without examining the results of other factors. The time-dose interaction was examined next. If the time-dose interaction was statistically identified,

linear contrasts tested the time-dose interaction for the comparisons of each dose group to the control group. A Bonferroni correction was applied to the alpha level to compensate for the multiple comparisons with the control group. This correction controls the experiment-wise error rate. The corrected comparison-wise alpha level of 0.02 was reported so direct comparison could be made to the p-values generated.
Terminal body weight and organ weight (absolute and relative, excluding ovaries, uterus, epididymides and testes) data were evaluated using a two-way ANOVA with the factors of sex and dose. Differences between the groups were primarily detected by the dose factor. For these parameters the first examination was whether the sex-dose interaction was significant at alpha = 0.05; if it was, a one-way ANOVA was done separately for each sex. Comparisons of individual dose groups to the control group were made with a Dunnett's test only when a statistically significant dose effect existed (alpha = 0.05). Dunnett's test corrects for multiple comparisons to the control and the experiment-wise error rate was reported.
Ovaries, uterus, epididymides and testes weights were analyzed using a one-way ANOVA. If significant dose effects (alpha = 0.05) were determined in the one-way ANOVA then separate doses were compared to controls using a Dunnett's test.
Food consumption data were evaluated by Bartlett's test for equality of variances. Exploratory data analysis was performed by a parametric analysis of variance (ANOVA) and if significant at alpha = 0.05, followed by Dunnett's test, alpha = 0.05. The experiment-wise alpha level was reported.
Descriptive statistics only were reported for body weight gains, RBC indices and differential RBC counts. Statistical outliers were identified by a sequential test, and routinely excluded from food consumption statistics. Other outliers may have been excluded only for documented, scientifically sound reasons. DCO incidence scores were qualitatively evaluated.
Because numerous measurements were statistically compared to the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results are consistent with other biological and pathological findings and historical control values.

C. METHODS:

1. **Observations:** Twice daily a cage-side examination was conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Detailed clinical observations (DCO) were conducted pre-exposure, then on a weekly basis, and included cage-side, hands-on and open-field observations which were recorded categorically or using explicitly defined scales.

2. **Body Weight:** Individual body weights were measured on a weekly basis throughout the study period.

3. **Food Consumption and Compound Intake:** Food consumption was measured once a week throughout the study period. From the body weight and food intake data, the actual test material intake per animal (mg/kg bw/day) was calculated.

4. **Ophthalmoscopic Examination:** Eyes of all animals were examined prior to study initiation and during the week prior to necropsy using indirect ophthalmoscopy, and during necropsy using a moistened glass slide pressed to the cornea.

5. **Hematology & Clinical Chemistry:** Blood samples were taken pre-exposure, midway through the

treatment period and on the day of necropsy from all animals via the jugular vein. for hematology and clinical chemistry analysis. Animals were fasted prior to collection. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

a. Hematology

✓	*Hematocrit (HCT)*	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	*Mean corpusc. volume (MCV)*
✓	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
✓	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	*Blood creatinine*
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium		Globulins
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (also SGPT)		
✓	Serum aspartate amino-transferase (also SGOT)		
	Sorbitol dehydrogenase		
✓	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. **Urinalysis**: Urine samples were collected from each dog prior to study initiation, midway through the treatment period and during week 13. Animals were placed in individual metabolism cages with urine collection vessels. and urine was collected overnight for a period of approximately 16 hours. Animals were food fasted during collection.

✓	Appearance	✓	Glucose
✓	Volume	✓	Ketones
✓	Specific gravity / osmolality	✓	Bilirubin
✓	pH	✓	Blood / blood cells
✓	Sediment (microscopic)		Nitrate
✓	Protein	✓	Urobilinogen

7. **Sacrifice and Pathology**: At study termination, animals were tranquilized by SC administration of acepromazine. subsequently sacrificed by an IV overdose of sodium pentobarbital and exsanguination,

and then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Oral tissues and tongue	✓	Aorta	✓	Brain
				✓	
✓	Salivary glands	✓	Heart	✓	Periph.nerve
		✓			
✓	Esophagus	✓	*Bone marrow*	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
				✓	
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)
		✓			
✓	Jejunum	✓	*Thymus*		GLANDULAR
		✓			
✓	Ileum			✓	Adrenal gland
				✓	
✓	Cecum		UROGENITAL		Lacrimal gland
✓	Colon	✓	*Kidneys*	✓	Mammary gland
		✓			
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
				✓	
✓✓	Liver	✓	Testes	✓	Thyroids
		✓		✓	
✓	Gall bladder	✓	Epididymides		OTHER
		✓			
✓	Pancreas	✓	Prostate	✓	Bone
	RESPIRATORY		Seminal vesicle	✓	Skeletal muscle
✓	Trachea	✓	*Ovaries with oviducts*	✓	Skin
		✓			
✓	Lung	✓	Uterus	✓	All gross lesions and masses
		✓			
✓	Nose	✓	Cervix		
✓	Pharynx				
✓	Tonsils				

The (✓) tissues were examined from all animals in the control, 0.15%, 0.75% and 3.0% groups.

II. RESULTS

A. *Observations*:

1. Mortality: All animals survived the duration of the study period.

2. Clinical Observations: There were no treatment-related findings.

B. Body Weight and Weight Gain: There was no treatment-related effect on body weight or body weight gain.

C. Food Consumption and Compound Intake:

1. **Food Consumption**: There were no treatment-related findings.

2. **Compound Consumption**: Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

3. **Food Efficiency**: Not conducted.

D. **Ophthalmoscopic Examination**: There were no treatment-related findings.

E. **Blood Analyses**:

1. **Hematology**: There were no treatment-related findings.

2. **Clinical Chemistry**: There were no treatment-related findings.

F. **Urinalysis**: There were no treatment-related findings.

G. **Sacrifice and Pathology**:

1. **Organ Weights**: Refer To Tables 2 and 3. Absolute and relative liver weights were statistically significantly higher in the 3.0% group, both sexes. The study author did not consider this finding to be treatment-related since the liver weights were within or near the historical control range of 13-week dietary dog studies, the absolute liver weights of the control group animals were lower than historical control values, there were no alterations in liver-specific clinical chemistry parameters and there were no corresponding microscopic changes to the liver. However, the historical control data were obtained from only 2 studies and only the mean liver weights were submitted (i.e., the range of individual liver weights were not included). In addition, since the absolute and relative liver weights for both sexes in the 3.0% group were statistically significantly higher than the liver weights for the concurrent control groups, the PMRA reviewer does not consider it appropriate to rule out the increase in liver weights as unrelated to treatment.
There were no other treatment-related findings.

TABLE 2 - Liver Weights of Males [a], absolute (g) and relative to bw (g/100 g)

	Dose (%)			
	0.00	0.15	0.75	3.00
Liver - absolute	236.15±12.523	224.15±21.580	226.23±14.533	266.35±16.815*
- relative to body weight	2.477±0.317	2.540±0.282	2.377±0.246	2.850±0.293*

[a] Data obtained from pages 113 and 114 in the study report; n=4.
[b] Historical control data were obtained from two 13-week dog studies, dated June 1998 and June 1999; mean values for absolute weight were 257.33±37.332 and 268.10±43.895; and for relative weight were 2.357±0.085 and 2.338±0.161.
* statistically significantly different from control, $p < 0.05$

TABLE 3 - Liver Weights of Females [a], absolute (g) and relative to bw (g/100 g)

	Dose (%)			
	0	0.15	0.75	3.00
Liver - absolute	192.43±37.706	213.98±27.374	196.98±16.056	236.00±17.689*
- relative to body weight	2.415±0.266	2.596±0.295	2.499±0.107	2.761±0.167*

[a] Data obtained from pages 115 and 116 in the study report; n=4.
[b] Historical control data were obtained from two 13-week dog studies, dated June 1998 and June 1999; mean values for absolute weight were 204.43±38.895 and 228.40±35.898; and for relative weight were 2.830±0.298 and 2.276±0.147.
* statistically significantly different from control, $p < 0.05$

2. **Gross Pathology:** There were no treatment-related findings.

3. **Microscopic Pathology:** Refer to Table 4. All animals in the 3.0% group exhibited slight diffuse hyperplasia and hypertrophy of the mucosal epithelium of the stomach. The mucosal hyperplasia was characterized by increased numbers of mucous cells and chief cells in the fundus of the stomach. Mucous cell hyperplasia was also noted in the pylorus of the stomach. Hypertrophy of mucous cells, characterized by increased cytoplasmic volume, was most prominent in mucous cells of the pylorus. There was no accompanying degeneration, necrosis or inflammation of the mucosa of the stomach. There were no other findings considered to be related to treatment with XDE-750.

Table 4 - Selected Histopathological Findings in the Stomach[a]

	Dose, %			
	0	0.15	0.75	3.00
Slight, diffuse mucosal hyperplasia/hypertrophy				
Males	0	0	0	4
Females	0	0	0	4

[a] Data obtained from page 131 in the study report; n=4.

III. DISCUSSION

A. Investigators' Conclusions: "There were no treatment-related effects on body weights, feed consumption, ophthalmologic and clinical observations, organ weights or clinical pathology parameters. A treatment-related microscopic effect was noted in the stomachs of all males and females given 3.0% XDE-750. The effect was characterized by slight, diffuse hyperplasia and hypertrophy of mucous cells, and slight, diffuse hyperplasia of chief cells in the gastric mucosa. There were no treatment-related effects in males or females given 0.15 or 0.75% XDE-750. The no-observed-effect level (NOEL) in male and female Beagle dogs following 13-weeks of dietary exposure was 0.75% XDE-750 (282/232 mg/kg/day)."

B. Reviewer Comments: Male and female beagle dogs were fed test diets containing XDE-750, purity 94.5%, at concentrations of 0, 0.15%, 0.75% or 3.0% (equal to 0, 54.5, 282 or 1070 mg/kg bw/day for males, and 0, 52.7, 232 or 929 mg/kg bw/day for females) for a period of 13 weeks, 4 dogs per sex per group. There were no treatment-related effects on body weights, food consumption, ophthalmologic and clinical observations or clinical pathology parameters. Liver weights were higher in the 3.0% group, both sexes. However, in the absence of any corresponding clinical chemistry findings or gross/microscopic changes to the liver, this effect was considered adaptive rather than adverse. Treatment-related

histopathological changes to the stomach were observed for all animals in the 3.0% group, manifest as slight diffuse hyperplasia and hypertrophy of the mucosal epithelium.
Based on the results of this study, the LOAEL was determined to be 3.0% (equal to 1070 mg/kg bw/day for males and 929 mg/kg bw/day for females) based on stomach histopathology. The NOAEL was 0.75% (equal to 282 mg/kg bw/day for males and 232 mg/kg bw/day for females).

C. **Study Deficiencies:** No scientific deficiencies were noted in the study.

005100 (15)

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:**
Fungicide/Herbicide Toxicological Evaluation
Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C)
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Repeat-dose (28-day) Dermal Toxicity - Rat ; OPPTS 870.3250 (rodent); OECD 410.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Stebbins, K.E., et al (2002) XDE-750: 28-Day Dermal Toxicity Study in Fischer 344 Rats. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011219, December 23, 2002. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a repeat-dose dermal toxicity study (MRID 46235626), XDE-750, purity 94.5%, was applied to the shaved skin of 10 Fischer 344 rats/sex/dose at dose levels of 0, 100, 500 or 1000 mg/kg bw/day, 6 hours/day, 7 days/week for 28 days.
There were no adverse, treatment-related effects on mortality, clinical signs, ophthalmology, body weight, food consumption, clinical chemistry, hematology, organ weights or gross pathology. The only finding was slight epidermal hyperplasia observed in the 500 and 1000 mg/kg bw/day study groups, males only.
The LOAEL for systemic toxicity could not be determined since there were no adverse, treatment-related systemic effects observed at any dose level tested. The NOAEL is 1000 mg/kg bw/day (limit dose).
For males, the dermal LOAEL is 500 mg/kg bw/day based on slight epidermal hyperplasia. The NOAEL is 100 mg/kg bw/day. For females, the dermal LOAEL could not be determined since there were no adverse, treatment-related effects observed at any dose level tested. The NOAEL is 1000 mg/kg bw/day.

This dermal toxicity study in the rat is acceptable and satisfies the guideline requirement for a repeat-dose dermal toxicity study (OPPTS 870.3250, rat) ; OECD 410 in rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-750

Description:	Technical: tan powder.
Lot/Batch #:	F-0031-143; TSN102319
Purity:	94.5% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** 0.5% aqueous methylcellulose

3. **Test Animals:**

Species:	Rat	
Strain:	Fischer 344	
Age/weight at study initiation:	~ 8 weeks of age. Males, 177.1 g to 207.7 g; Females, 115.9 g to 140.9 g.	
Source:	Charles River Laboratories Inc., Raleigh, NC.	
Housing:	Individually in suspended stainless steel cages with wire-mesh floors.	
Diet:	LabDiet, Certified Rodent Diet #5002, in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	20.9-22.2°C
	Humidity:	45.9-54.9%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 16 days.	

B. STUDY DESIGN:

1. **In Life Dates:** May 16, 2002 to June 14, 2002.

2. ***Animal Assignment:*** *Animals were stratified by pre-exposure body weight then randomly assigned to* the test groups noted in Table 1 using a computer program.

TABLE 1 - Study Design

Test Group	Dose (mg/kg bw/d)	# Male	# Female
Control	0	10	10
Low	100	10	10
Mid	500	10	10
High	1000	10	10

3. **Dose Selection Rationale:** The high-dose of 1000 mg/kg bw/day (limit test dose level) was chosen based on the results of the acute oral and dermal studies (LD_{50} values were > 5000 mg/kg bw); the 4-week dietary toxicity study in rats (NOAEL of 1000 mg/kg bw/day); and the 13-week dietary toxicity study in rats (NOAEL of 500 mg/kg bw/day). The remaining dose levels were expected to provide dose-

response data for any treatment-related effects(s) observed in the high-dose group and to ensure definition of a no-observed-effect level (NOEL).

4. **Preparation and Treatment of Animal Skin:** On the day prior to the first application and weekly thereafter, the area on the back (i.e., shoulders to the wing of the ilium and half way down the flank on each side) of each animal was clipped free of hair. The test material was applied to an area that was at least 10% of the total body surface. The applied quantities of the test substance were adjusted weekly to individual animal body weights. The test material was directly applied to the shaved test area as a suspension in 0.5% aqueous methylcellulose, 4 mL/kg. The test sites were covered with a gauze dressing and non-absorbent cotton, which were secured by wrapping with an elastic bandage. The dressings were removed after ~ 6 hours and the application areas were cleaned with a water-dampened towel. Animals were dosed for 6 hours a day, 7 days a week, for 28 days.
Rats in the control group were exposed to the vehicle using the same procedure as described for the treated rats.

4. **Statistics:** Means and standard deviations were calculated for all continuous data. Body weights, food consumption, organ weights, urine specific gravity, clinical chemistry data, coagulation and appropriate hematologic data were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, exploratory data analyses were performed by a parametric or nonparametric analysis of variance (ANOVA). If significant at alpha = 0.05, the ANOVA was followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons to the control. The experiment-wise alpha level was reported for these 2 tests. DCO incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group. Data collected at different time points were analyzed separately. Descriptive statistics only were reported for body weight gains, RBC indices and differential RBC counts. Statistical outliers were identified by a sequential test, but routinely were excluded only from food consumption statistics. Outliers, if identified, were excluded from other analyses only for documented, scientifically sound reasons.
Because numerous measurements were statistically compared to the same group of animals, the overall false positive rate (Type I errors) will be greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results are consistent with other biological and pathological findings and historical control values.

C. **METHODS:**

1. **Observations:** Animals were observed at least twice daily for mortality and morbidity, and twice daily for clinical signs of toxicity. A detailed clinical examination was conducted once a week. The application sites were examined on a weekly basis for signs of local dermal irritation and were evaluated according to the laboratory's modification of the scoring system recommended by the OECD (1992).

2. **Body Weight:** Individual animals were weighed on a weekly basis throughout the study period.

3. **Food Consumption:** Individual food consumption was determined on a weekly basis, calculated from the weight of the offered diet at the beginning of a specific week and its difference to the re-weight amount at the end of the week. Mean food consumption was reported as g food/animal/day.

4. **Ophthalmoscopic Examination:** An ophthalmological examination was conducted on each animal prior to study initiation and prior to termination using indirect ophthalmoscopy.

5. **Hematology & Clinical Chemistry:** Blood samples were taken at study termination from all surviving animals via the orbital sinus, after animals were anesthetized with CO_2 asphyxiation, for hematology and clinical chemistry analysis. Animals were fasted overnight prior to collection. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

a. **Hematology**

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
✓	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
✓	(Prothrombin time)		

b. **Clinical Chemistry**

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium (K)		Globulins
✓	Sodium (NA)	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (AP)	✓	Total serum protein
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (ALT/also SGPT)		
✓	Serum aspartate amino-transferase (AST/also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase		

6. **Urinalysis:** The week prior to necropsy, individual urine samples were collected for a 16-hour period from all animals (non-fasted) using metabolism cages. The CHECKED (✓) parameters were examined.

✓	Appearance	✓	Glucose
	Volume	✓	Ketones
✓	Specific gravity / osmolality	✓	Bilirubin
✓	pH	✓	Blood
	Sediment (microscopic)		Nitrate
✓	Protein	✓	Urobilinogen

7. **Sacrifice and Pathology:** At study termination, animals were anesthetized by CO_2 asphyxiation,

sacrificed by decapitation, then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Tongue and oral tissues	✓	Aorta	✓	Brain
				✓	
✓	Salivary glands	✓	Heart	✓	Peripheral nerve
		✓			
✓	Esophagus	✓	*Bone marrow*	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic nerve)
		✓			
✓	Jejunum	✓	Thymus		GLANDULAR
		✓			
✓	Ileum			✓	Adrenal gland
				✓	
✓✓	Cecum		UROGENITAL	✓	Lacrimal/Harderian glands
✓	Colon	✓	Kidneys	✓	Mammary gland
		✓			
✓	Rectum		Urinary bladder	✓	Parathyroid
✓✓	Liver	✓	Testes	✓	Thyroid
		✓			
	Gall bladder	✓	Epididymides	✓	Auditory sebaceous glands
		✓			
✓	Pancreas	✓	Prostate	✓	Coagulating glands
	RESPIRATORY		Seminal vesicles		OTHER
✓	Trachea	✓	Ovaries with oviducts	✓	Bone
		✓			
✓	Lung	✓	Uterus	✓	Skeletal muscle
		✓			
✓	Nose	✓	Cervix	✓	Skin (treated & untreated areas)
✓	Pharynx	✓	Vagina	✓	All gross lesions and masses
✓	Larynx				

The (✓) tissues were examined from all animals in the control and 1000 mg/kg bw/day groups. In addition, the liver, lungs, kidneys, dermal test site, skin adjacent to the dermal test site and relevant gross lesions were examined for all animals in the 100 and 500 mg/kg bw/day groups.

II. RESULTS

A. Observations:

1. **Mortality:** All animals survived the duration of the study period.

2. **Clinical Observations:** There were no overt, clinical signs of treatment-related toxicity.

3. **Dermal Irritation:** One male in the 1000 mg/kg bw/day group was observed to have scabs on the dermal test site on day 28, which were attributed to the clipping procedure. There were no other findings noted in any animal at any dose level during the study period.

B. Body Weight and Body Weight Gain: There were no treatment-related findings.

C. Food Consumption:

1. Food Consumption: There were no treatment-related findings.

2. Food Efficiency: Not conducted.

D. Ophthalmoscopic Examination: There were no treatment-related findings.

E. Blood Analyses:

1. Hematology: There were no treatment-related findings.

2. Clinical Chemistry: There were no treatment-related findings.

F. Urinalysis: There were no treatment-related findings.

G. Sacrifice and Pathology:

1. Organ Weight: a) **Males:** Absolute and relative (to body weight) full cecum weights were increased at all dose levels, attaining statistical significance in the 100 and 500 mg/kg bw/day groups. However, this finding was not statistically significant in the 1000 mg/kg bw/day group, there was no dose-response relationship, empty cecal weights were comparable amongst all groups and there were no microscopic changes noted at histopathological examination. Hence, this was not considered to be an adverse, treatment-related finding.

TABLE 2 - Selected Organ Weights of Male Rats[a], absolute (g) and relative to bw (% bw)

	Dose (ppm)			
	0	100	500	1000
Cecum, full - absolute	3.596±0.495	4.232±0.385*	4.364±0.842*	4.123±0.458
- relative to body weight	1.681±0.243	1.963±0.175*	1.979±0.327*	1.882±0.113
Cecum, empty - absolute	1.111±0.130	1.045±0.138	1.169±0.119	1.160±0.113
- relative to body weight	0.519±0.064	0.486±0.073	0.532±0.045	0.532±0.071

[a] Data obtained from page 60 in the study report; n=10.
* statistically significantly different from control, $p < 0.05$

b) **Females:** There were no treatment-related findings.

2. Gross Pathology: There were no treatment-related findings. The only observation was a slight increase in the incidence of animals with mottling or necrosis of the hepatic papillary process in the 1000 mg/kg bw/day group. However, this was attributed to the compressive effects of the elastic bandages used to hold the test material in place.

Table 3 - Selected Gross Pathological Findings in the Liver of Male and Female Rats[a]

	Dose (ppm)			
Liver Findings	0	100	500	1000
Mottled, papillary process, focal:				
Males	1	0	1	1
Females	2	0	0	2
Mottled, papillary process, focally extensive:				
Males	0	0	0	1
Females	0	0	0	1
Necrosis, papillary process, focal:				
Males	0	0	0	2
Females	0	0	0	0
Necrosis, papillary process, focally extensive:				
Males	0	0	0	0
Females	0	0	0	1

[a] Data obtained from page 66 in the study report; n=10.

3. **Microscopic Pathology:** The only treatment-related finding was an increased incidence of epidermal hyperplasia (severity: slight), at the dermal test site for males in the 500 and 1000 mg/kg bw/day groups. There was a slight increase in the number of animals exhibiting infarction or necrosis in the liver in the 1000 mg/kg bw/day group. However, the severity of these findings was very slight to slight, and were attributed to the compressive effects of the elastic bandages used to hold the test material in place.

Table 4 - Selected Histopathological Findings in Male and Female Rats[a]

	Dose (ppm)			
	0	100	500	1000
Dermal Findings				
Slight epidermal hyperplasia:				
Males	0	0	2	3
Females	0	0	0	0
Hepatic Findings				
Infarct, papillary process, focally extensive:				
Males	0	0	0	1
Females	1	0	0	3
Inflammation, chronic, focal:				
Males	1	1	3	5
Females	2	1	0	3
Inflammation, chronic, papillary process, multifocal:				
Males	0	0	0	1
Females	0	0	0	1
Inflammation, chronic, portal, focal:				
Males	0	0	0	0
Females	0	1	0	0
Necrosis, hepatocyte, periportal, focal:				
Males	0	0	0	1
Females	0	0	0	0

	Dose (ppm)			
Necrosis and inflammation, papillary process, multifocal:				
Males	1	0	1	0
Females	0	0	0	0
Necrosis and inflammation, hepatocyte, focal:				
Males	1	1	0	3
Females	0	0	0	0

* Data obtained from pages 74, 75, 79 and 80 in the study report; n=10.

III. DISCUSSION

A. **Investigators' Conclusions:** The only treatment-related effect was slight epidermal hyperplasia at the dermal test site of 2 males given 500 mg/kg bw/day, and 3 males given 1000 mg/kg bw/day. The slight epidermal hyperplasia was indicative of minimal irritation in response to dermal application of XDE-750. Under the conditions of this study, the no-observed-effect level (NOEL) for Fischer 344 rats following 28-days of 6 hour/day dermal exposure to XDE-750 was the targeted concentration of 100 mg/kg bw/day for males and 1000 mg/kg bw/day for females. The NOEL for systemic effects was 1000 mg/kg bw/day for both males and females."

B. **Reviewer Comments:** The reviewer agrees with the study author's conclusions.

C. **Study Deficiencies:** No scientific deficiencies were noted in the study.

1/2 108

005100
(11)

PMRA Sub. No. 2004-0789/DOW AMINOPYRALID/AMD — ~ PROTECTED ~ — 1-Year Dog Study / 1 DACO 4.3.2 / OECD IIA 5.3.4

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** for
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: One-Year Dietary Toxicity Study in Dogs; OPPTS 870.4100; OECD 452.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Stebbins, K.E., et al (2003) XDE-750: One-Year Dietary Toxicity Study in Beagle Dogs. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 021027. September 10, 2003. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 46235627), XDE-750 Technical, purity 94.5%, was administered to 4 Beagles per sex per group in the diet at concentrations of 0, 0.03%, 0.30% or 3.0% or 0, 300, 3000, and 30000 ppm (equal to 0, 9.9, 99.2 or 967 mg/kg BW/day for males, and 0, 9.2, 93.2 or 1038 mg/kg bw/day for females) for a period of one year. There were no treatment-related effects on body weights, food consumption, ophthalmologic and clinical observations or clinical pathology parameters. Liver weights were higher in the 3.0% group, both sexes, with very slight hepatocyte hypertrophy noted at histopathological examination. These liver effects were considered to be adaptive rather than adverse. In the stomach, gross examination revealed diffuse thickening of the stomach mucosa for females in the 3.0% group. Histopathological changes in the stomach were observed for all animals in the 3.0% group, manifest as slight diffuse hyperplasia and hypertrophy of the mucosal epithelium of the stomach, slight lymphoid hyperplasia of the gastric mucosa and very slight/slight chronic mucosal inflammation.
The LOAEL is 3.0% or 30000 ppm (equal to 967 mg/kg bw/day for males and 1038 mg/kg bw/day for females), based on stomach histopathology. The NOAEL is 0.30% or 3000 ppm (equal to 99.2 mg/kg bw/day for males and 93.2 mg/kg bw/day for females).

This study in dogs is acceptable and satisfies the guideline requirement for a 1-year oral toxicity study in dogs (OPPTS 870.4100, OECD 452).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-143; TSN102319
Purity:	94.5% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5007 Certified Canine Lab Diet).

3. **Test Animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	7 months of age; Males, 6.951 kg to 10.021 kg; Females, 5.860 kg to 8.527 kg.	
Source:	Marshall Farms USA, Inc., North Rose, NY.	
Housing:	Individually in pens, ~ 1 m wide x 2 m long x 1.5 m high with plastic coated grid floors.	
Diet:	Purina Certified Canine Lab Diet # 5007 in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	21-24°C
	Humidity:	41.4-70%
	Air changes:	12-15/hr
	Photoperiod:	12hrs dark/12 hrs light
Acclimation period:	27 to 33 days.	

B. STUDY DESIGN:

1. **In Life Dates:** April 10, 2002 to April 21, 2003.

2. **Animal Assignment:** Animals were stratified by pre-exposure body weight and then randomly assigned to the test groups noted in Table 1 using a computer program. Littermates were not included in the same treatment groups.

TABLE 1 - Study Design

Test Group	Conc. in Diet (%)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	0/0	4	4
Low	0.03	9.9/9.2	4	4
Mid	0.3	99.2/93.2	4	4
High	3.0	967/1038	4	4

3. **Dose Selection Rationale:** The high-dose level was chosen based on the results of the 13-week study in Beagles, and was expected to produce clear evidence of treatment-related microscopic alterations of the stomach and possibly an increase in liver weights. The remaining dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group and to

ensure definition of a no-observed-effect level.

4. **Diet Preparation and Analysis:** Fresh diets were prepared at least every two weeks. The high-dose diet was prepared by mixing test material with control diet to obtain the desired percent of test material in the diet. Mid-dose diets were prepared by serially diluting the high-dose level diet with control diet, and the low-dose diets were prepared by diluting the mid-dose level diet with control diet. Stability of the test material in diet for up to 21 days was demonstrated in the 13-week dietary toxicity study in dogs (Study No. 001239R), and so additional stability analyses were not conducted for the 1-year dog study. Homogeneity of mixing was determined for the low-dose female test diets and the high-dose male test diets prior to study initiation, and during study weeks 17, 35 and 51. Actual test material concentration in the diet was determined for all dose levels from test diets prepared just prior to study initiation, and during study weeks 15, 35 and 51.

Results - Homogeneity Analysis: Individual samples of 4 separate batches of the 0.03% female test diets ranged from 88.3% to 95.0%, 90.3% to 95.3%, 87.7% to 92.3% and 75.0% to 88.7% of the nominal concentration, respectively.
Individual samples of 4 separate batches of the 3.0% male test diets ranged from 94.0% to 104.7%, 91.7% to 101.7%, 91.7% to 98.3% and 99.7% to 108.3% of the nominal concentration, respectively.

Stability Analysis: (Results are from the 13-week dog dietary study, Study No. 001239R). The actual concentration of XDE-750 in the 0.15% and 3.0% test diets, expressed as percentage of the nominal concentration, were as follows:

Dose (%)		
Storage Interval	**0.15**	**3.0**
Day 0	93.3%	105.0%
Day 14	102.0%	112.7%
Day 21	100.7%	116.3%

Concentration Analysis: Individual samples of test diets at all dose levels ranged from 81.7% to 103% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Means and standard deviations were calculated for all continuous data. All parameters examined statistically were first evaluated by Bartlett's test for equality of variances. If the results from Bartlett's test were significant at alpha = 0.01, then the data for the parameter may have been subjected to a transformation to obtain equality of the variances. The transformations that were examined were the common log, the inverse and the square root. The data were reviewed and an appropriate form of the data was selected. The selected form of the data was then subjected to the appropriate parametric analysis as described below.

In-life body weight, urine specific gravity, hematologic parameters (excluding RBC indices and differential WBC) coagulation and clinical chemistry parameters were evaluated using a repeated measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex and dose. In the RM-

ANOVA, differences between the groups were detected primarily by the time-dose interaction. The first examination in the RM-ANOVA was the time-sex-dose interaction. If significant at alpha = 0.02, the analysis was repeated separately for each sex without examining the results of other factors. The time-dose interaction was examined next at alpha = 0.05. If the time-dose interaction was statistically identified, linear contrasts tested the time-dose interaction for the comparisions of each dose group to the control group. A Bonferroni correction was applied to the alpha level to compensate for the multiple comparisons with the control group. This correction controls the experiment-wise error rate. The corrected comparison-wise alpha level of 0.02 was reported so direct comparison could be made to the p-values generated.

Terminal body weight and organ weight (absolute and relative, excluding ovaries, uterus, prostate, epididymides and testes) and urine volume data were evaluated using a two-way ANOVA with the factors of sex and dose. Differences between the groups were primarily detected by the dose factor. For these parameters the first examination was whether the sex-dose interaction was significant at alpha = 0.05; if it was, a one-way ANOVA was done separately for each sex. Comparisons of individual dose groups to the control group were made with a Dunnett's test only when a statistically significant dose effect existed (alpha = 0.05). Dunnett's test corrects for multiple comparisons to the control and the experiment-wise error rate was reported.

Ovaries, uterus, epididymides and testes weights were analyzed using a one-way ANOVA. If significant dose effects (alpha = 0.05) were determined in the one-way ANOVA then separate doses were compared to controls using a Dunnett's test.

Food consumption data were evaluated by Bartlett's test for equality of variances. Exploratory data analysis was performed by a parametric analysis of variance (ANOVA) and if significant at alpha = 0.05, followed by Dunnett's test, alpha = 0.05. The experiment-wise alpha level was reported. Descriptive statistics only were reported for body weight gains, RBC indices and differential WBC counts. Statistical outliers were identified by a sequential test, and routinely excluded from food consumption statistics. Other outliers may have been excluded only for documented, scientifically sound reasons. DCO incidence scores were qualitatively evaluated.

Because numerous measurements were statistically compared to the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results are consistent with other biological and pathological findings and historical control values.

C. METHODS:

1. Observations: Once a day a cage-side examination was conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function and behavior were evaluated. In addition, mortality, moribundity and availability of food and water were assessed twice daily. Detailed clinical observations (DCO) were conducted pre-exposure, then on a weekly basis, and included cage-side, hands-on and open-field observations that were recorded categorically or using explicitly defined scales.

2. Body Weight: Individual body weights were measured on a weekly basis for the first 13 weeks of the study, then every 4 weeks thereafter.

3. Food Consumption and Compound intake: Food consumption was measured on a weekly basis for the first 13 weeks of the study, then every 4 weeks thereafter. From the body weight and food intake data, the actual test material intake per animal (mg/kg bw/day) was calculated.

4. Ophthalmoscopic Examination: Eyes of all animals were examined prior to study initiation and during the week prior to necropsy using indirect ophthalmoscopy, and during necropsy using a moistened glass slide pressed to the cornea.

5. Hematology and Clinical Chemistry: Blood samples were taken pre-exposure, during week 14, 26 (males), 27 (females) and on the morning of necropsy from all animals via the jugular vein, for hematology and clinical chemistry analysis. Animals were fasted prior to collection. The hematological and clinical chemistry parameters marked with an (✓) in Tables (a) and (b), respectively, were examined.

a. Hematology

✓	Hematocrit (HCT)	✓	Leukocyte differential count
✓	Hemoglobin (HGB)	✓	Mean corpuscular HGB (MCH)
✓	Leukocyte count (WBC)	✓	Mean corpusc. HGB conc.(MCHC)
✓	Erythrocyte count (RBC)	✓	Mean corpusc. volume (MCV)
✓	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
✓	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
✓	Calcium	✓	Albumin
✓	Chloride	✓	Blood creatinine
	Magnesium	✓	Blood urea nitrogen
✓	Phosphorus	✓	Total Cholesterol
✓	Potassium		Globulins
✓	Sodium	✓	Glucose
	ENZYMES	✓	Total bilirubin
✓	Alkaline phosphatase (ALK)	✓	Total serum protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
✓	Serum alanine amino-transferase (also SGPT)		
✓	Serum aspartate amino-transferase (also SGOT)		
	Sorbitol dehydrogenase		
✓	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis: Urine samples were collected from each dog prior to study initiation, during week 14, week 26 (males), week 27 (females) and on the morning of necropsy. Animals were placed in individual metabolism cages with urine collection vessels, and urine was collected overnight for a period of approximately 16 hours. Animals were food fasted during collection.

✓	Appearance	✓	Glucose
✓	Volume	✓	Ketones
✓	Specific gravity / osmolality	✓	Bilirubin
✓	pH	✓	Blood / blood cells
✓	Sediment (microscopic)		Nitrate
✓	Protein	✓	Urobilinogen

7. **Sacrifice and Pathology:** At study termination, animals were tranquilized by SC administration of acepromazine, subsequently sacrificed by an IV overdose of sodium pentobarbital and exsanguination, and then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
✓	Oral tissues and tongue	✓	Aorta	✓	Brain
				✓	
✓	Salivary glands	✓	Heart	✓	Periph.nerve
		✓			
✓	Esophagus	✓	Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
				✓	
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)
		✓			
✓	Jejunum	✓	Thymus		GLANDULAR
✓	Ileum			✓	Adrenal gland
				✓	
✓	Cecum		UROGENITAL		Lacrimal gland
✓	Colon	✓	Kidneys	✓	Mammary gland
		✓			
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
				✓	
✓✓	Liver	✓	Testes	✓	Thyroids
		✓		✓	
✓	Gall bladder	✓	Epididymides		OTHER
		✓			
✓	Pancreas	✓	Prostate	✓	Bone
		✓			
	RESPIRATORY		Seminal vesicle	✓	Skeletal muscle
✓	Trachea	✓	Ovaries with oviducts	✓	Skin
		✓			
✓	Lung	✓	Uterus	✓	All gross lesions and masses
		✓			
✓	Nose	✓	Cervix		
✓	Pharynx				
✓	Tonsils				

The (✓) tissues were examined from all animals in the control, 0.03%, 0.30% and 3.0% groups.

II. RESULTS

A. Observations:

1. Mortality: All animals survived the duration of the study period.

2. Clinical Observations: There were no treatment-related findings.

B. Body Weight and Weight Gain: Refer to Table 2. For males, there was no treatment-related effect on body weight/body weight gain. Although mean final body weight in the 0.03% group was ~9% lower than the control group value, this finding was not statistically significant and a similar effect was not seen in the 0.3% or 3% groups, where final body weights were comparable to the control group value. For females, final body weight was lower in the 3.0% group by ~9%, and was considered to be treatment-related by the study author. However, this finding was not statistically significant, and the magnitude of the effect is similar to that seen for males in the 0.03% group. Hence, although it is possible this could reflect a marginal, treatment-related effect, the reviewer does not consider this to be an adverse effect, and most likely reflects normal body weight variation.

TABLE 2 - Mean Body Weights (BW) and Body Weight Gains (BWG)[a]

%	0.00	0.03	0.30	3.00
MALES				
Initial BW	8.722±0.864	8.609±1.274 (98.7%)	8.836±0.844 (101.3%)	8.920±0.650 (102.3%)
Final BW	10.731±1.212	9.772±1.504 (91.1%)	10.508±0.836 (97.9%)	10.598±0.387 (98.8%)
BWG Wk 1	0.008±0.079	0.069±0.331	-0.002±0.162	0.124±0.177
BWG Wk 1-13	0.739±0.811	0.077±0.733	0.392±0.773	0.725±0.835
BWG Wk 13-25[b]	0.737	1.020	1.051	0.784
BWG Wk 25-52[b]	0.533	0.066	0.228	0.169
Overall BWG, Wk 1-52	2.009±1.463	1.162±1.413 (57.8%)	1.671±0.619 (83.2%)	1.678±0.657 (83.5%)
FEMALES				
Initial BW	6.948±0.963	7.085±0.814 (102.0%)	6.914±0.563 (99.5%)	7.119±1.063 (102.5%)
Final BW	8.638±1.650	8.263±0.944 (95.7%)	8.739±1.040 (101.2%)	7.831±1.488 (90.7%)
BWG Wk 1	0.088±0.235	-0.092±0.091	0.248±0.134	0.064±0.123
BWG Wk 1-13	0.719±0.504	0.365±0.185	0.762±0.245	0.527±0.211
BWG Wk 13-25[b]	0.315	0.378	0.664	0.057
BWG Wk 25-52[b]	0.656	0.435	0.399	0.127
Overall BWG Wk, 1-52	1.691±0.799	1.179±0.503 (69.7%)	1.825±0.507 (107.9%)	0.712±0.425 (42.1%)

[a] Data obtained from pages 66 to 73 in the study report.
[b] Calculated by the PMRA reviewer; standard deviations not available.

C. **Food Consumption and Compound Intake:**

1. **Food Consumption:** There were no treatment-related findings.

2. **Compound Consumption:** Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

3. **Food Efficiency:** Not conducted.

D. **Ophthalmoscopic Examination:** There were no treatment-related findings.

E. **Blood Analyses:**

1. **Hematology**: There were no treatment-related findings.

2. **Clinical Chemistry:** There were no treatment-related findings.

F. **Urinalysis:** There were no treatment-related findings.

G. **Sacrifice and Pathology:**

1. **Organ Weights:** Refer to Tables 3 and 4. The only treatment-related findings were statistically significantly increased relative liver weights in the 3.0% group, both sexes, and, although not statistically significant, increased absolute liver weight for males in the 3.0% group.

TABLE 3 - Liver Weights of Males [a], absolute (g) and relative to bw (g/100 g)

	Dose (%)			
	0.00	0.03	0.30	3.00
Liver - absolute	253.98±23.289	240.10±9.279	272.58±25.074	307.28±34.380
- relative to body weight	2.443±0.067	2.553±0.360	2.643±0.161	2.970±0.289*

[a] Data obtained from pages 144 and 145 in the study report; n=4.
* statistically significantly different from control, $p < 0.05$

TABLE 4 - Liver Weights of Females [a], absolute (g) and relative to bw (g/100 g)

	Dose (%)			
	0.00	0.03	0.30	3.00
Liver - absolute	236.78±22.896	225.53±21.486	240.60±46.696	241.25±23.374
- relative to body weight	2.914±0.255	2.818±0.266	2.837±0.520	3.224±0.416*

[a] Data obtained from pages 146 and 147 in the study report; n=4
* statistically significantly different from control, $p < 0.05$
** statistically significantly different from control, $p < 0.01$.

2. **Gross Pathology:** Refer to Table 4. The only finding considered to be related to treatment was diffuse thickening of the stomach mucosa, observed in 2 females in the 3.0% group.

Table 5 - Selected Gross Pathological Findings in the Stomach[a]

	Dose, %			
	0	0.03	0.30	3.0
Diffuse thickening of the mucosa				
Males	0	0	0	0
Females	0	0	0	2

[a] Data obtained from page 152 in the study report; n=4.

3. Microscopic Pathology: Refer to Table 4. All animals in the 3.0% group exhibited treatment-related findings in the stomach, manifest as slight diffuse hyperplasia and hypertrophy of the mucosal epithelium of the stomach, slight lymphoid hyperplasia of the gastric mucosa and very slight/slight chronic mucosal inflammation. The hyperplasia and hypertrophy were characterized by increased numbers of mucous cells in the cardia, fundus and pylorus of the stomach. The mucous cells were also increased in size, especially in the pylorus where the cytoplasm of affected cells was distended with mucous. The inflammatory effect consisted of lymphocytes and plasma cells accumulating in the lamina propria of the cardia, fundus and/or pylorus. The lymphoid hyperplasia was characterized by numerous prominent lymphoid follicles with germinal centres, located in the cardia, fundus and/or pylorus.
The only other finding considered to be related to treatment was very slight hypertrophy of the centrilobular to midzonal hepatocytes observed in 2 males and 2 females in the 3.0% group.

Table 6 - Selected Histopathological Findings in the Stomach and Liver[a]

	Dose, %			
	0	0.03	0.30	3.0
Stomach				
Slight, diffuse mucosal hyperplasia/hypertrophy				
Males	0	0	0	4
Females	0	0	0	4
Slight, lymphoid hyperplasia of the mucosa				
Males	0	0	0	3
Females	0	0	0	4
Very slight/diffuse chronic mucosal inflammation				
Males	0	0	0	4
Females	0	0	0	4
Liver				
Very slight hepatocyte hypertrophy				
Males	0	0	0	2
Females	0	0	0	2

[a] Data obtained from pages 157 and 161 in the study report; n=4

III. DISCUSSION

A. Investigators' Conclusions: "There were no treatment-related effects on feed consumption, ophthalmogical and clinical observations, or clinical pathology parameters. High-dose (3.0% XDE-750) females had treatment-related lower final body weights and body weight gains. High-dose animals had

treatment-related, statistically identified higher relative liver weights averaged across both sexes, relative o controls. In addition, there was a treatment-related non-statistically identified increase in the mean absolute liver weight of high-dose males, relative to controls. The liver weight alterations corresponded to very slight hypertrophy of centrilobular to midzonal hepatocytes in 2 males and 2 females from the high-dose males and females. Treatment-related microscopic effects were noted in the stomachs of all high-dose males and females. The stomach effects consisted of slight, diffuse mucosal hyperplasia and hypertrophy, very slight or chronic mucosal inflammation and slight lymphoid hyperplasia of the gastric mucosa. There were no treatment-related effects in males or females given 0.03 or 0.3% XDE-750. The no-observed-effect level (NOEL) in male and female Beagle dogs following one year of dietary exposure was 0.3% XDE-750, which corresponded to 99 and 93 mg/kg/day, respectively."

B. Reviewer Comments: Male and female beagle dogs were fed test diets containing XDE-750, purity 94.5%, at concentrations of 0, 0.03%, 0.30% or 3.0% (equal to 0, 9.9, 99.2 or 967 mg/kg bw/day for males, and 0, 9.2, 93.2 or 1038 mg/kg bw/day for females) for a period of one year, 4 dogs per sex per group. There were no treatment-related effects on body weights, food consumption, ophthalmologic and clinical observations or clinical pathology parameters. Liver weights were higher in the 3.0% group, both sexes, with corresponding hepatocyte hypertrophy (very slight) noted at histopathological examination. In the stomach, gross examination revealed diffuse thickening of the stomach mucosa for females in the 3.0% group. These liver effects were considered to be adaptive rather than adverse. Histopathological changes in the stomach were observed for all animals in the 3.0% group, manifest as slight diffuse hyperplasia and hypertrophy of the mucosal epithelium of the stomach, slight lymphoid hyperplasia of the gastric mucosa and very slight/slight chronic mucosal inflammation.
Based on the results of this study, the LOAEL was determined to be 3.0% (equal to 967 mg/kg bw/day for males and 1038 mg/kg bw/day for females) based on stomach histopathology. The NOAEL was 0.30% (equal to 99.2 mg/kg bw/day for males and 93.2 mg/kg bw/day for females).

C. Study Deficiencies: No scientific deficiencies were noted in the study.

005100 (12)

PMRA Sub. No. 2004-0789/DOW AMINOPYRALID/AMD — ~ PROTECTED ~ — Mouse Carcinogenicity Study / 1 DACO 4.4.3 / OECD IIA 5.5.3

PMRA Primary Reviewer: Brenda MacDonald, DVM
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: for
Date Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)
Signature:
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Feeding Study in Mice; OPPTS 870.4200; OECD 451.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Stebbins K.E., et al (2003) XDE-750: Oncogenicity Dietary Study in CD-1 Mice. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011163, December 19, 2003. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 46235628), purity 94.5%, was administered to 50 CD-1 mice/sex/dose in the diet at dose levels of 0, 50, 250 or 1000 mg/kg bw/day (equal to 0, 50.2, 251 or 1000 mg/kg bw/day for males, and 0, 50.9, 252 or 1010 mg/kg bw/day for females) for 18 months.

There were no treatment-related effects on mortality, clinical signs, ophthalmology, body weight/body weight gain, food intake, food efficiency, hematology, organ weights, gross pathology or histopathological examination. The only oncogenic finding was an increased incidence of pulmonary bronchiolo-alveolar carcinomas in the 1000 mg/kg bw/day group, males only. Historical control data for this tumour type indicated that the incidence observed in this study was within the normal range. Thus the slight increased in bronchiolo-alveolar tumours in the high-dose males was not considered to be treatment related.

The systemic LOAEL could not be determined since there were no adverse, treatment-related findings. The NOAEL is 1000 mg/kg bw/day (equal to 1000 mg/kg bw/day for males and 1010 mg/kg bw/day for females).

This carcinogenicity study in the mouse is acceptable and satisfies the guideline requirement for a carcinogenicity study (OPPTS 870.4200); OECD 451 in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-125; TSN102095
Purity:	95.4% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5002 Certified Rodent Lab Diet).

3. **Test Animals:**

Species:	Mouse	
Strain:	CD-1	
Age/weight at study initiation:	6 weeks of age: Males, 27.2 g to 33.9 g; Females, 20.8 g to 27.7 g.	
Source:	Charles River Laboratories, Inc., Portage, MI.	
Housing:	Individually housed in suspended stainless steel cages with wire-mesh floors.	
Diet:	Purina Certified Rodent Lab Diet #5002 in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	21.6-22.2°C
	Humidity:	44.9-62.1%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days.	

B. STUDY DESIGN:

1. **In Life Dates:** November 14, 2001 to May 20, 2003.

2. **Animal Assignment/Dose Levels:** Animals were stratified by body weight and then randomly assigned using a computer program to the test groups noted in Table 1.

TABLE 1 - Study Design

Test Group	Conc. in Diet (mg/kg bw/day)	Dose to Animal (mg/kg bw/day)	# Males	# Females
Control	0	0/0	50	50
Low	50	50.2/50.9	50	50
Mid	250	251/252	50	50
High	1000	1000/1010	50	50

3. **Dose Selection:** The limit test of 1000 mg/kg bw/day was chosen as the high-dose based on the results of a 90-day mouse study in which adverse effects were not observed. The intermediate- and low-dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group. The low dose was also expected to be a no-observed-effect level (NOEL).

4. Diet Preparation and Analysis: Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were mixed approximately bi-monthly for the first 90 days of the study, and at approximately monthly intervals thereafter. Initial concentrations of test material in the diet were calculated from historical control body weights and food consumption data. Subsequently, the concentrations of the test material in the feed were adjusted weekly for the first 90 days of the study and at approximately monthly intervals thereafter, based upon the most recent body weight and food consumption data. Stability of the test material in diet for up to 21 days was demonstrated in the 4-week dietary toxicity study in mice (MRID 46235624). Additional stability analyses were evaluated for at least 34 days at concentrations of 0.00258% and 5% (premix) to enable monthly mixing procedures. Homogeneity of mixing was determined for the low-dose female diets and high-dose male diets prior to study initiation and during months 3, 8, 13 and 17. Actual test material concentration in the diet was determined for all dose levels from test diets prepared just prior to study initiation and during months 3, 8, 13 and 17.

Results - Homogeneity Analysis: Individual samples of 5 separate batches of the 50 mg/kg bw/day female test diets ranged from 92.2% to 121.7%, 104.7% to 117.8%, 90.8% to 103.2%, 88.3% to 98.8% and 96.1% to 103.0% of the nominal concentration, respectively. Individual samples of 5 separate batches of the 1000 mg/kg bw/day male test diets ranged from 99.2% to 107.3%, 97.0% to 129.8%, 96.2% to 102.5%, 82.8% to 92.7% and 95.0% to 100.4% of the nominal concentration, respectively.

Stability Analysis: i) Results from the 4-week mouse dietary study, MRID 46235624. The actual concentration of XDE-750 in the 10 and 1000 mg/kg bw/day test diets, expressed as percentage of the nominal concentration, were as follows:

Dose (mg/kg bw/day)		
Storage Interval	**10**	**1000**
Day 0	95.6%	109.0%
Day 21	100.6%	116.8%

ii) Results from stability analysis conducted for the current study, at dose levels of 0.025% and 5%, expressed as percentage of the nominal concentration:

Dose (%)		
Storage Interval	**0.0258**	**5**
Day 0	80.6%	103.5%
Day 10	99.6%	83.2%
Day 23	109.7%	100.0%
Day 35	122.5%	84.6%

Concentration Analysis: The range of values for the actual concentrations of XDE-750 in the test diets, and the overall mean values, expressed as percentage of the nominal concentrations, were as follows:

	Dose (mg/kg bw/day)			
	0	50	250	1000
Actual concentration, ppm				
Range of values	None detected	45.3 to 62.5	241.3 to 290.0	857.0 to 1060.0
Mean value		53.3	263.8	976.0
% of target concentration				
Range of values	None detected	90.5% to 125.0%	96.5% to 116.0%	85.7% to 106.0%
Mean value		106.5%	105.5%	97.6%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5.. **Statistics:** Means and standard deviations were calculated for all continuous data. Body weights, feed consumption, organ weights, and total white blood cell counts were evaluated by Bartlett's test for equality of variances (alpha = 0.01). Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance (ANOVA). If the ANOVA was significant at alpha = 0.05, it was followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons to the control. The experiment-wise alpha level of alpha = 0.05 was reported for Dunnett's test and Wilcoxon Rank-Sum test. DCO incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group at alpha = 0.05. Descriptive statistics only (means and standard deviations) were reported for body weight gains, feed efficiency, and differential WBC counts. Statistical outliers were identified by a sequential test (alpha = 0.02), but routinely excluded only from feed consumption and feed efficiency analyses. Outliers were excluded from other analyses only for documented, scientifically sound reasons.
Statistical analyses were conducted on body weight, feed consumption, and organ weight data throughout this oncogenicity study. However, such data near termination of an oncogenicity rodent study were confounded by a spectrum of geriatric changes, the presence of spontaneous tumors, secondary effects from tumors, and terminal changes prior to death. As a result of these changes, statistical tests were of questionable value and extra caution should be applied in interpreting the statistical result.
Gross pathologic observations were tabulated and considered in the interpretation of final histopathologic data, but were not evaluated statistically. The cumulative incidence of histopathologic observations for all animals scheduled for the terminal sacrifice was used in the statistical analysis. For tissues where all animals in all dose groups were scheduled to be examined, the incidences of specific histopathologic observations were first tested for deviation from linearity (alpha = 0.01) using ordinal spacing of the doses. If linearity was not rejected the data was then tested for a linear trend using the Cochran-Armitage Trend test. If the trend was statistically significant at alpha = 0.02, or if significant deviation from linearity was found, incidences for each dose group were compared to that of the control group using a pairwise Chi-square test with Yates' continuity correction (alpha = 0.05, two-sided). For tissues which were evaluated from all control-dose and high-dose animals, but only from selected animals in the intermediate-dose groups, statistical analysis consisted of the pairwise comparisons of control and high dose using the pairwise Chi-square test with Yates' continuity correction (alpha = 0.05, two-sided). Rare tumors, those with a background incidence of less than or equal to 1%, were considered significant in the Chi-square test with Yates' continuity correction at alpha = 0.10, two-sided. Differences in mortality patterns were tested by the Gehan-Wilcoxon procedure for all animals scheduled for terminal sacrifice. If a significant effect was identified for all dose groups (alpha = 0.05) then individual analyses were run comparing each dose to control and were Bonferroni corrected to compensate for the multiple

comparisons with the control group. The experiment-wise alpha level was reported. Since there were statistically-identified differences in median survival times among the treatment groups for females, mortality adjusted analyses for tumors were used.

C. METHODS:

1. Observations: A cage-side examination was conducted at least once a day. This examination was performed with the animals in their cages and include, but were not limited to: activity, repetitive behavior, vocalization, incoordination/lameness, injury, neuromuscular function (convulsion, fasciculation, tremor, twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), fecal consistency, and fecal/urinary quantity. At least twice daily, usually at the beginning and end of each day, all animals were observed for morbidity and mortality, and the availability of feed/water. Detailed clinical observations (DCO) were conducted on all animals on days 7 for females and 8 for males (baseline) and at approximately monthly intervals for months 1-9. During months 9-12, month 17 and at study termination, the first 10 surviving animals/sex/dose group were evaluated for DCO parameters. These examinations were performed at approximately the same time each examination day according to an established format, which included cage-side, hand-held and open-field observations that were recorded categorically or using explicitly defined scales (scored). Additionally, all animals were examined for palpable masses once per month, starting at month 6 and continuing through month 18. The time of onset, location, dimensions, appearance and progression of each palpable mass was recorded.

2. Body Weight: All mice were weighed during the pre-exposure period, weekly during the first 13 weeks of the study and then at approximately monthly intervals, thereafter.

3. Food Consumption and Compound Intake: Feed consumption data were collected weekly during the first 13 weeks of the study and then at approximately monthly intervals for all animals by weighing feed containers at the start and end of a measurement cycle. Mean daily dietary consumption was calculated as g food/kg body weight/day. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (expressed as mg/kg bw/day and PPM) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic Examination: The eyes of all animals was examined by a veterinarian pre-exposure and prior to the scheduled necropsy using indirect ophthalmoscopy. Eyes were also examined by a prosector during the necropsy using a moistened glass slide pressed to the cornea.

5. Hematology: Blood smears were made from all surviving, non-fasted, animals, while under carbon dioxide anesthesia, via sample collection from the pedal vein (12 months) or orbital sinus at terminal sacrifice (18 months). Blood from moribund animals was obtained from the pedal vein or tail. Blood smears were not obtained from animals that died spontaneously. A white blood cell count and differential white blood count were determined from all animals in the treated and control groups at terminal sacrifice. A differential white blood cell count, as derived from the blood smears, was not determined from animals that were moribund due to the absence of effects at 18 months.

Clinical Chemistry: Not conducted.

6. Urinalysis: Not conducted.

7. Sacrifice and Pathology: At study termination, animals were anesthetized by inhalation of carbon dioxide, sacrificed by decapitation, and then necropsied. The CHECKED (✓) tissues were collected and preserved in neutral, phosphate-buffered 10% formalin, then prepared for histological examination. In addition, the (✓✓) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC/HEMAT.		NEUROLOGIC
✓	Oral tissues	✓	Aorta	✓	Brain
				✓	
✓	Salivary glands	✓	Heart	✓	Periph.nerve
		✓			
✓	Esophagus	✓	Bone marrow	✓	Spinal cord (3 levels)
✓	Stomach	✓	Lymph nodes	✓	Pituitary
✓	Duodenum	✓	Spleen	✓	Eyes (optic n.)
		✓			
✓	Jejunum	✓	Thymus		GLANDULAR
✓	Ileum			✓	Adrenal gland
				✓	
✓	Cecum		UROGENITAL	✓	Lacrimal/Harderian glands
✓	Colon	✓	Kidneys	✓	Mammary gland
		✓			
✓	Rectum	✓	Urinary bladder	✓	Parathyroids
✓✓	Liver	✓	Testes	✓	Thyroids
		✓			
✓	Gall bladder	✓	Epididymides		Auditory sebaceous glands
		✓			
✓	Pancreas	✓	Prostate	✓	Coagulating glands
	RESPIRATORY	✓	Seminal vesicle		OTHER
✓	Trachea	✓	Ovaries and oviducts	✓	Bone
		✓			
✓	Lung	✓	Uterus	✓	Skeletal muscle
		✓			
✓	Nasal tissues	✓	Cervix	✓	Skin
	Pharynx	✓	Vagina	✓	All gross lesions and masses
✓	Larynx				

The (✓) tissues were examined from all animals in the control and 1000 mg/kg bw/day groups, and all animals that died or were sacrificed in a moribund condition. In addition, liver, lungs, kidneys and relevant gross lesions were examined for all animals in the 50, 2500 and 500 mg/kg bw/day groups.

II. RESULTS

A. Observations:

1. Mortality: The mortality rates at the end of the study were 38, 32, 34, and 42% for males in the control, 50, 250, and 1000 mg/kg bw/day groups, respectively, and 16, 34, 30, and 42% for females in the control, 50, 250, and 1000 mg/kg bw/day groups, respectively. The incidence of mortality in females given 50 or 1000 mg/kg/day was statistically identified as increased, relative to controls. There were no

statistically identified differences in the incidence of mortality in females given 250 mg/kg/day, nor in males at any dose level. The most common cause of death in high-dose females was nephropathy. However, the overall incidence and severity of nephropathy was not increased in males or females from any dose group. Therefore, the increased number of high-dose females that died or were euthanized moribund was interpreted to be unrelated to treatment. The statistically identified increase in mortality of females given 50 mg/kg bw/day was interpreted to not be treatment related because of the lack of a dose response, and the absence of any treatment-related histopathologic effects at this dose level.

Table 2 - Mortality Incidence and Survival Rates for Male and Female Mice[a]

	Dose (mg/kg bw/day)			
	0	**50**	**250**	**1000**
Mortality, Sacrificed: Males	6	7	7	10
Females	3	10	7	10
Mortality, Found dead: Males	13	8	10	11
Females	5	7	8	10
Mortality, Accidental: Males	0	1	0	0
Females	0	0	0	1
Survival rate, at 12 months: Males	88%	86%	84%	82%
Females	98%	90%	94%	90%
Survival rate, at 18 months: Males	62%	68%	66%	58%
Females	84%	66%*	70%	58%*

[a] Data extracted from pages 85, 86 and 512 to 1416 of the study report, and from the individual animal data; n=50.
* Statistically significantly different from control, $p<0.05$.

2. Clinical Observations: There were no overt, clinical signs of treatment-related toxicity.

B. Body Weight: There was no treatment-related effect on body weight or body weight gain.

C. Food Consumption and Compound Intake:

1. Food Consumption: There were no treatment-related findings.

2. Compound Consumption (time-weighted average): Based on food consumption, the nominal dietary concentrations and body weight, the doses expressed as mean daily mg test substance/kg body weight during the study period are presented in Table 1.

3. Food Efficiency: Calculated feed efficiencies were highly variable within and between dose levels and were likely a reflection of the normal variability in feed consumption and body weight gain. There were no treatment-related effects on feed efficiency.

D. Ophthalmoscopic Examination: There were no treatment-related findings.

E. Blood Analyses:

1. Hematology: There were no treatment-related findings.

2. Clinical Chemistry: Not conducted.

F. Urinalysis: Not conducted.

G. Sacrifice and Pathology:

1. Organ Weight:
Males: There were no treatment-related findings.

Females: Refer to Tables 3 and 4. There were no treatment-related findings in organ weights of male or female mice at any dose level tested. The only statistically identified alterations in organ weights were elevated mean absolute and relative ovary weights of mice given 50 or 250 mg/kg bw/day. The elevated ovary weights from these dose levels were reflective of an increase in the incidence of individual animals with grossly observed fluid-filled ovarian cysts. These cysts were recorded as dilatation of the ovarian bursa or as hematocysts at gross necropsy, and were not drained prior to weighing so that the structural integrity of the ovaries was maintained for histological examination. Microscopic evaluation of the ovaries from all females revealed no treatment-related increases in the number of animals with ovarian cysts or hematocysts. The mean absolute and relative ovary weights of mice given 1000 mg/kg bw/day were also higher than controls, but not statistically identified. The elevated mean ovary weights of mice given 1000 mg/kg/day were reflective of one female with markedly distended fluid-filled cysts of both ovaries. If the ovary weight (6.798 grams) of this individual animal was excluded from statistical analysis, the mean absolute and relative ovary weights of mice given 1000 mg/kg bw/day would have been comparable (0.119 grams) to the controls. The alterations in ovary weights were interpreted to not be treatment related because of the lack of a dose response, and the absence of any histopathologic ovarian effects. The higher mean ovarian weights in the 50 and 250 mg/kg bw/day groups were interpreted to be reflective of the variability in the size of ovarian cysts or hematocysts. If the ovarian weights of animals from the scheduled terminal necropsy that had grossly observed dilatation of the ovarian bursa or hematocysts were excluded from analysis, the ovarian weights of all dose levels were comparable to controls.

TABLE 3 - Ovary Weights, all ovaries[a], absolute (g) and relative to bw (g/100 g)

	Dose (mg/kg bw/day)			
	0 (n=42)	**50 (n=33)**	**250 (n=35)**	**1000 (n=29)**
Ovaries - absolute	0.161±0.342	0.236±0.279*	0.359±0.566*	0.349±1.259
- relative	0.438±0.938	0.628±0.734*	0.976±1.611*	0.886±3.055

[a] Data obtained from page 115 in the study report.
* Statistically significantly different from control, $p<0.05$.

TABLE 4 - Ovary Weights, excluding animals with dilated ovarian bursa or hematocyst[a], absolute (g) and relative to bw (g/100 g)

	Dose (mg/kg bw/day)			
	0 (n=33)	50 (n=15)	250 (n=16)	1000 (n=22)
Ovaries - absolute	0.043±0.030	0.052±0.054	0.049±0.027	0.047±0.058
- relative	0.116±0.077	0.136±0.143	0.128±0.069	0.123±0.148

[a] Data obtained from page 116 in the study report.
* Statistically significantly different from control, $p<0.05$.

2. Gross Pathology:

Males: There were no treatment-related findings.

Females: Refer to Table 5. In the 1000 mg/kg bw/day, there was an increased incidence of pale kidneys. Most of the animals with pale kidneys had moderate or severe nephropathy. Other gross observations that were more frequent in females given 1000 mg/kg bw/day consisted of decreased amount of body fat, the presence of hemolyzed blood in the gastrointestinal tract, pulmonary atelectasis and perineal soiling. These gross observations were interpreted to reflect debility and/or stress of the animals that died spontaneously or were euthanized moribund, and not primary treatment-related alterations. Females given 50, 250 or 1000 mg/kg bw/day had an increased incidence of dilatation of the ovarian bursa. Microscopic evaluation of the ovaries revealed that most of the fluid-filled ovarian structures were not dilated bursas, but rather, were ovarian cysts derived from anovulatory follicles, or from epithelial cords of the interstitium of the ovary. The increased incidence of dilatation of the ovarian bursa in females given 50, 250 or 1000 mg/kg bw/day was interpreted to not be treatment-related because of the lack of a dose response, and the absence of any histopathologic ovarian effects. Ovarian cysts are a common spontaneous alteration in aging female mice.

TABLE 5 - Selected Gross Pathological Findings

	Dose (mg/kg bw/day)			
Finding	0	50	250	1000
Kidneys, pale; bilateral	5	6	7	13
Decreased body fat	4	5	8	9
Hemolyzed blood in GIT	1	5	1	10
Atelectesis	0	0	2	4
Perineal soiling	2	5	3	8
dilatation, Ovarian Bursa, any Symmetry	11	25	27	16

[a] Data obtained from pages 118 to 134 in the study report; n=50.

3. Microscopic Pathology:

a) Non-neoplastic: There were no treatment-related statistically identified histopathologic effects in males or females at any dose level. The overall incidence of nephropathy in treated mice from all modes of death (spontaneous death, moribund, and scheduled terminal sacrifice) was comparable to controls at all dose levels. However, in females given 1000 mg/kg bw/day (high-dose) that died spontaneously or were euthanized moribund, there was an increased incidence of moderate or severe nephropathy as a contributory factor of their deaths. There were 11 mice of early death or moribund status with

nephropathy at the 1000 mg/kg bw/day dose level, versus 4, 2, and 4 mice of early death or moribund status with nephropathy at the 0, 50, or 250 mg/kg bw/day dose levels, respectively. The increased incidence of moderate or severe nephropathy in high-dose females that died or were euthanized moribund was interpreted to be unrelated to treatment, because the overall incidence and severity of nephropathy was not increased in males or females from any dose group. Spontaneous nephropathy is a common age-related degenerative alteration of mice.

b) Neoplastic: Refer to Table 6. There was an increased incidence of bronchiolo-alveolar carcinoma in the lungs in the 1000 mg/kg bw/day group, males only. Historical control data for this tumor type indicated that the incidence observed in this study was within the normal range. Thus the slight increased in bronchiolo-alveolar tumors in the high-dose males was not considered to be treatment related. There were no other differences between treated animals and controls in the number of tumor bearing mice (benign and/or malignant) or the incidence of any specific tumor type in any organ.

TABLE 6 - Incidence of Pulmonary Bronchiolo-Alveolar Tumors in Male Mice[a]

	Dose (mg/kg bw/day)			
	0	50	250	1000
Adenoma; primary, incidental	9	11	9	6
Adenoma; two, primary, incidental	0	0	0	1
Adenoma; three, primary, incidental	0	1	0	1
Carcinoma; malignant with metastasis, primary, fatal	0	0	0	1
Carcinoma; malignant without metastasis, primary, incidental	2	1	2	5

[a] Data obtained from page 150 in the study report; n=50.

III. DISCUSSION

A. Investigators' Conclusions: "There were no treatment-related effects in males at any dose level. Females given 1000 mg/kg/day (high-dose) had a statistically identified increase in mortality which was interpreted to be treatment related. The most common cause of death in high-dose females was nephropathy. However, the overall incidence and severity of nephropathy was not increased in males or females from any dose group. Therefore, the increased number of high-dose females that died or were euthanized moribund due to nephropathy was interpreted to be unrelated to treatment. There were no other treatment-related effects in females at any dose level. No increase in neoplasms was observed in either male or female mice at any dose level indicating that XDE-750 did not have an oncogenic potential under the conditions of this study. The NOEL for males was 1000 mg/kg/day and for females was 250 mg/kg/day."

B. Reviewer Comments: Male and female CD-1 mice were fed test diets containing technical XDE-750, purity 94.5%, at dose levels of 0, 50, 250 or 1000 mg/kg bw/day (equal to 0, 50.2, 251 or 1000 mg/kg bw/day for males, and 0, 50.9, 252 or 1010 mg/kg bw/day for females) for up to 18 weeks, 50 mice/sex/group.
There were no treatment-related effects on mortality, clinical signs, ophthalmology, body weight/body weight gain, food intake, food efficiency, hematology, organ weights, gross pathology or

histopathological examination. The only oncogenic finding was an increased incidence of pulmonary bronchiolo-alveolar carcinomas in the 1000 mg/kg bw/day group, males only. Historical control data for this tumor type indicated that the incidence observed in this study was within the normal range. Thus the slight increased in bronchiolo-alveolar tumors in the high-dose males was not considered to be treatment related.

The systemic LOAEL could not be determined since there were no adverse, treatment-related findings observed at any dose level tested. The NOAEL was 1000 mg/kg bw/day (equal to 1000 mg/kg bw/day for males and 1010 mg/kg bw/day for females).

C. **Study Deficiencies:** No scientific deficiencies were noted in the study.

005100
(13)

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:**
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rat; OPPTS 870.3700; OECD 414.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Carney, E.W., et al (2001) XDE-750: Oral Gavage Developmental Toxicity Study in CD Rats. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011061, November 20, 2001. Unpublished. MRID NO. 46235629

Tornesi, B., et al (2001) XDE-750: Developmental Toxicity Probe Study in CD Rats. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 001234, May 4, 2001. Unpublished MRID NO. 46235630

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46235629), XDE-750, 94.5%, in 0.5% Methocel A4M, was administered to pregnant CD rats by gavage at dose levels of 0, 100, 300 or 1000 mg/kg bw/day, 25 females per group, from days 6 through 20 of gestation. There were no treatment-related effects on mortality, body weight and body weight gain, food intake, organ weights, gross pathology or reproductive parameters.

The maternal LOAEL could not be determined since there were no treatment-related effects observed at any dose level tested. The NOAEL is 1000 mg/kg bw/day.

There were no treatment-related developmental nor teratogenic effects noted at any dose level tested.

The developmental LOAEL could not be determined an the developmental NOAEL is 1000 mg/kg bw/day.

The developmental toxicity study in the rat is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-143; TSN102319
Purity:	94.5% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** 0.5% Methocel A4M

3 **Test Animals:**

Species:	Rats, time-mated females.	
Strain:	CD (Crl:CD(SD)IGS BR)	
Age/weight at study initiation:	10 to 11 weeks of age. 200 g to 250 g.	
Source:	Charles River Laboratories Inc., Portage, Michigan.	
Housing:	Individually in suspended stainless steel cages with wire-mesh floors.	
Diet:	LabDiet Certified Rodent Diet #5002, in meal form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±2°C
	Humidity:	49-51%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Four days prior to dosing.	

B. PROCEDURES AND STUDY DESIGN

1. **In Life Dates:** May 8, 2001 to June 13, 2001.

2. **Mating:** Sexually mature virgin females were mated with males of the same strain, 1:1 ratio. Confirmation of mating was determined by the presence of a copulation plug and was designated as day 0 of gestation. Day 0 body weights were provided by CTL; rats arrived at the study laboratory on gestation day 1 or 2.

3. **Animal Assignment:** Animals were randomly assigned to dose groups as indicated in Table 1 based upon gestation day 0 body weights and using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of dosing.

TABLE 1 - Animal Assignment

Dose (mg/kg bw/day)	0	100	300	1000
# Females	25	25	25	25

4. Dose Selection Rationale: The dose levels were chosen based on the results of the probe study (MRID 46235633), in which there was no evidence of maternal toxicity nor any adverse effects on embryo/fetal survival at dose levels up to and including 1000 mg/kg bw/day. Hence, the limit dose of 1000 mg/kg bw/day was chosen as the high dose level. The lower dose levels were selected to provide dose response data for any potential toxicity among the high-dose group animals.

5. Dosage Preparation and Analysis: Dosing solutions were freshly prepared in an aqueous solution of 0.5% Methocel A4M. Further details were not provcided.
Stability of the test substance in suspension was established to be at least 17 days in a concurrent probe study in rabbits (Study #DR-0293-9028-020), at dose levels of 250 and 1000 mg/kg bw/day.
Homogeneity of the test material in suspension was evaluated at 100 and 1000 mg/kg bw/day from the first lot of test preparations. Samples of test material suspensions at all dose levels were taken from the first preparations and were analyzed for actual test material concentration.

Results - Homogeneity Analysis: Individual samples of the 100 and 1000 mg/kg bw/day suspensions ranged from 92.0% to 104.8%, and 89.2% to 103.2% of the nominal concentrations, respectively.

Stability Analysis: Stability of the test substance in suspension was established in study #DR-0293-9028-020). The actual concentration of XDE-750 in the 250 and 1000 mg/kg bw/day formulations, after 17 days storage, expressed as percentage of the initial concentration, were 102.6% and 100.4%, respectively.

Concentration Analysis: Individual samples of the 100, 300 and 1000 mg/kg bw test suspensions fell between 97% and 98% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage Administration: All doses were administered once daily by oral gavage, on gestation days 6 through 20, at a dose volume of 4 mL/kg bw/day. Dose volumes were adjusted daily based on individual body weights.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: Clinical examinations were conducted daily throughout the study period, which included hand-held evaluation of the skin, fur, mucous membranes, respiration, nervous system function, unusual swelling or masses and animal behavior. In addition, at least once each day cage-side examinations were conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Body weights were measured on day 0, day 3, daily during the dosing period and on day 21. Individual food consumption was recorded for days 3-6, 6-9, 9-12, 12-15, 15-18 and 18-21. Dams were sacrificed on day 21 by inhalation of carbon dioxide, and were then necropsied. At that time, the uterus, liver and kidneys were removed and weighed. The uterus was examined for the number of implantation sites, number and location of live and dead fetuses, and number, location and classification of resorption sites. Resorption sites were classified as either early or late based on the presence (late resorption) or absence (early resorption) of grossly recognizable embryonic/fetal form. The ovaries were examined for the number of corpora lutea. Uteri which appeared non-pregnant were stained with a 10% aqueous solution of sodium sulfide and examined for evidence of early resorptions to determine pregnancy status.

Representative samples of liver, kidneys and gross lesions were preserved in neutral, buffered 10% formalin. Microscopic examination of tissues was not conducted.
Any animal that died, appeared moribund or showed indications of premature delivery was submitted for a complete necropsy by a veterinary pathologist. However, the liver, kidney and gravid uterine weights were not recorded, nor were the number of corpora lutea and the sex and body weight of fetuses from these animals recorded. Development of the conceptuses was determined to the extent possible by external examination (as appropriate for gestational age). The conceptuses were not examined for visceral or skeletal alterations. Following external examination, these conceptuses were discarded. Near term fetuses were euthanized by oral administration of sodium pentobarbital solution.

2. Fetal Evaluations: All fetuses were removed from the uterus, weighed and sexed, and then examined for external abnormalities. Fetuses were euthanized by oral administration of sodium pentobarbital solution. At least one-half of all the fetuses in each litter were chosen randomly via computer for visceral examination, conducted by dissection under a low power stereomicroscope for evidence of visceral alterations. The heads of these fetuses were removed, placed in Bouin's fixative and serially sectioned to allow for inspection of the eyes, brain, nasal passages, and tongue. The remaining fetuses not selected for visceral examination were skinned, eviscerated, preserved in alcohol, and double stained with Alcian Blue and Alizarin Red S for cartilage and bone according to methods derived from McLeod, Kimmel and Trammell, and Webb and Byrd. After staining, skeletons were macerated and cleared. A thorough evaluation of the fetal skeleton was conducted on the remaining fetuses not selected for visceral examination.

3. Classification of Fetal Findings:
a) Malformations: A permanent structural change that may adversely affect survival, development or function and/or which occurs at a relatively low incidence in the specific species/strain.
b) Variations: A divergence beyond the normal range of structural constitution that may not adversley affect survival or health.

D. DATA ANALYSIS

Maternal body weights, maternal body weight gains, organ weights (absolute and relative), fetal body weights and feed consumption were evaluated by Bartletts test (alpha=0.01) for equality of variances. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha=0.05, analysis by Dunnett's test (alpha=0.05) or the Wilcoxon Rank-Sum test (alpha=0.05) with Bonferroni's correction was performed, respectively. Frequency of pre-implantation loss, post-implantation loss (calculations shown below), resorptions per litter and resorptions per fetal population and fetal alterations were analyzed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implantations, and litter size was evaluated using a nonparametric ANOVA (alpha=0.05) followed by the Wilcoxon Rank-Sum test (alpha=0.05) with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test (alpha=0.05) with Bonferroni's correction. Fetal sex ratios were analyzed using a binomial distribution test. Non-pregnant females, females with resorptions only, or females found to be pregnant after staining of their uteri were excluded from the appropriate analyses. Statistical outliers were identified, using a sequential method (alpha=0.02), and excluded if justified by sound scientific reasons. Both Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control group to keep the experiment-wise alpha at 0.05. Both were reported at the experiment-wise alpha level. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final

interpretation of the data took into consideration statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

2. Indices: The following indices were calculated from caesarean section records of animals in the study:
a) Pre-implantation loss* = (# corpora lutea - implantations) x 100/# corpora lutea
b) Post-implantation loss* = (# implantations - live born pups) x 100/# implantations

* Note: Percent pre- and post-implantation losses were determined for each litter, followed by calculation of the mean of these litter values.

3. Historical Control Data: Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality: One female in the 300 mg/kg bw/day group was found dead on study day 10.
There were no abnormal findings at gross pathological examination, and all fetuses appeared normal.
This death was not considered to be related to treatment.
All remaining animals survived the duration of the study period.

2. Clinical Observations: There were no overt, clinical signs of treatment-related toxicity.

2. Body Weight: Refer to Table 2. There were no treatment-related effects on body weight or body weight gain at any dose level tested.

TABLE 2 - Maternal Body Weight and Body Weight Gain, (g)±SD[a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (25)	100 (24)	300 (23)	1000 (23)
Initial Body Weight	226.3±12.6	222.5±11.5	225.0±11.2	226.2±13.3
Pre-treatment (gain): Days - 0 to 6	38.0±8.3	34.9±5.4	36.3±7.5	37.5±8.5
Treatment (gain): Days - 6 to 21	138.2±19.3	139.0±16.8	139.8±15.7	137.7±13.5
Body weight gain, d. 0 to 21	176.2±20.8	173.9±20.3	176.5±17.4	175.1±19.7
Final body weight	303.6±19.3	298.1±18.6	306.3±16.2	300.4±25.5
Gravid Uterus Weight	98.80±15.74	98.35±12.26	97.16±13.31	100.88±14.24

[a] Data extracted from pages 30 and 31 of the study report

3. Food Consumption: There was no treatment-related effect on food intake at any dose level tested.

4. Organ Weights: Absolute and relative kidney and liver weights were comparable amongst all groups.

5. Gross Pathology: There were no treatment-related findings.

6. Caesarean Section Data: Refer to Table 3. There were no treatment-related findings.

TABLE 3 - Caesarean Section Observations[a]

Observation	Dose (mg/kg bw/day)			
	0	100	300	1000
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant Pregnancy Rate (%)	25 100.0%	24 96.0%	24 96.0%	23 92.0%
# Aborted	0	0	0	0
Gravid Uterus Weight, g	98.80±15.74	98.35±12.26	97.16±13.31	100.88±14.24
# with all dead/resorbed	0	0	0	0
# Corpora Lutea/Dam	18.8±4.1	16.9±4.1	18.6±6.1	17.9±3.3
# Implantations/Dam	13.4±1.9	13.2±1.2	13.1±1.7	13.3±1.9
Total # Litters	25	24	23	23
# Live Fetuses/Dam	12.7±2.4	12.5±2.0	12.4±2.0	12.7±2.0
Mean Fetal Weight (g): males females	5.87±0.37 5.51±0.34	5.93±0.38 5.64±0.32	5.87±0.31 5.57±0.27	5.96±0.26 5.65±0.32
Sex Ratio (% Male)	49%	52%	46%	51%
Resorptions/Litter	0.8±1.3	0.7±1.3	0.7±0.7	0.6±0.7
Preimplantation loss (%)	26.8±12.2	18.9±15.2	25.3±17.2	23.7±15.5
Postimplantation Loss (%)	6.0±10.3	5.7±10.3	5.3±6.0	4.6±5.6

[a] Data extracted from page 37 of the study report.

B. DEVELOPMENTAL TOXICITY

1. External Examination: Refer to Table 4. There were no treatment-related findings.
There were no external malformations noted at any dose level tested. The only variation was observed in one control fetus, manifest as generalized subdermal hematoma to the right side of the face.

TABLE 4 - External Examinations - Litter (fetal) incidence[a]

Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Litters (fetuses) examined	25 (317)	24 (300)	23 (286)	23 (292)
Total Malformations Total Variations	0 (0) 1 (1)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)

[a] Data extracted from pages 38 to 43 of the study report.

2. Visceral Examination: Refer to Table 5. There was no evidence of any treatment-related effects on the incidence of visceral malformations or variations at any dose level of XDE-750 tested. The specific malformations noted were as follows:

i) At 0 mg/kg bw/day: multilobed lung.
ii) At 100 mg/kg bw/day: no malformations;
iii) At 300 mg/kg bw/day: no malformations;
iv) At 1000 mg/kg bw/day: hemorrhage in the pericardial fluid.

TABLE 5 - Visceral Examinations - Litter (fetal) incidence[a]

Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Litters (fetuses) examined	25 (167)	24 (158)	23 (147)	23 (153)
Total Malformations	1 (1)	0 (0)	0 (0)	1 (1)
Total Variations	4 (4)	3 (3)	2 (3)	0 (0)

[a]Data extracted from pages 38 to 43, and pages 273 to 453 of the study report.

3. Skeletal Examination: Refer to Table 6. There was no evidence of any treatment-related effects on the incidence of skeletal malformations or variations at any dose level of XDE-750 tested. The specific malformations noted were as follows:

i) At 0 mg/kg bw/day: fused cervical centra, extra thoracic rib and extra thoracic vertebrae; missing caudal vertebrae, fused cervical centra and extra lumbar vertebrae; cervical and thoracic hemivertebrae, fused cervical centra, extra thoracic rib and vertebrae and fused thoracic vertebrae; sternoschisis; missing lumbar vertebrae.
ii) At 100 mg/kg bw/day: missing thoracic vertebrae amd missing thoracic rib.
iii) At 300 mg/kg bw/day: no malformations;
iv) At 1000 mg/kg bw/day: extra cervical rib.

TABLE 6 - Skeletal Examinations - Litter (fetal) incidence[a]

Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Litters (fetuses) examined	25 (150)	24 (142)	23 (139)	23 (139)
Total Malformations	3 (5)	1 (1)	0 (0)	1 (1)
Total Variations	12 (17)	10 (16)	7 (8)	11(20)

[a]Data extracted from pages 38 to 43, and pages 273 to 453 of the study report.

III. DISCUSSION

A. Investigators' Conclusions: "Oral gavage administration of XDE-750 up to and including the limit dose of 1000 mg/kg/day resulted in no maternal toxicity and no indications of embryonal/fetal toxicity or teratogenicity. Therefore, the NOEL for maternal toxicity and for developmental toxicity was 1000 mg/kg/day, the highest dose level tested."

B. Reviewer's Discussion:

1. **Maternal Toxicity:** Pregnant CD rats were dosed by oral intubation with XDE-750 technical, purity

94.5%, as a suspension in 0.5% Methocel A4M, at dose levels of 0, 100, 300 or 1000 mg/kg bw/day, 25 females per group, from day 6 to 20 of gestation, inclusive. There were no treatment-related effects on mortality, body weight and body weight gain, food intake, organ weights, gross pathology or reproductive parameters.

Maternal NOAEL = 1000 mg/kg bw/day.
The maternal LOAEL could not be determined since there were no treatment-related findings observed at any dose level tested.

2. Developmental Toxicity:

a. Deaths/Resorptions: The number of resorptions/dam and viable fetuses/dam were comparable between the concurrent control and treatment groups.

b. Altered Growth: There was no treatment-related effect on mean fetal body weights.

c. Developmental Variations: There were no treatment-related developmental variations noted at any dose level tested.

d. Malformations: There were no treatment-related developmental malformations noted at any dose level tested.

Developmental NOAEL = 1000 mg/kg bw/day
Developmental LOAEL could not be determined since there were no adverse, treatment-related findings.

C. Study Deficiencies: There were no scientific deficiencies noted in the study.

005100
(14)

PMRA Sub. No. 2004-0789/DOW AMINOPYRALID/AMD — ~ PROTECTED ~ — Rabbit Developmental Toxicity / 1 DACO 4.5.3 / OECD IIA 5.6.2.2

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:**
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509 **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rabbit; OPPTS 870.3700; OECD 414.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Marty, M.S., et al (2002) XDE-750: Oral Gavage Developmental Toxicity in New Zealand White Rabbits. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011047; 011047A, December 23, 2002. Unpublished.

Liberacki, A. et al (2001) - RANGE-FINDING SNDY MRID NO. 46235634

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

JR 2/2/06

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46235630), XDE-750, 94.5%, in 0.5% Methocel A4M, was administered to pregnant New Zealand White rabbits by gavage at dose levels of 0, 25, 100 or 250 mg/kg bw/day (Phase I) or 0, 500 or 750 mg/kg bw/day (Phase II), 26 females per group, from days 7 through 27 of gestation. Two animals in the 750 mg/kg bw/day group were sacrificed in a moribund condition (incoordinated gait, significant body weight losses, decreased food intake), which was considered to be treatment-related. In the 500 and 750 mg/kg bw/day groups, there was an increased incidence of incoordinated gait, which was more pronounced in the 750 mg/kg bw/day group. In most cases, the incoordination was transient, with complete resolution within two hours post-dosing, and did not appear to progressively worsen on subsequent days. The only other clinical observation was decreased amounts of feces observed in the 750 mg/kg bw/day group. There was a net loss in body weight in the 500 and 750 mg/kg bw/day groups during the first 3 days of the dosing period (days 7 to 10), with a corresponding decrease in food consumption, which was more pronounced in the 750 mg/kg bw/day group. The 750 mg/kg bw/day group was removed from the study on day 20 due to decreases in body weight gain on gestation days 7-10 and due to the severity of clinical signs. At necropsy, there was an increased incidence of pale kidneys and of ulcers/erosions in the glandular mucosa of the stomach in the 750 mg/kg bw/day group. The observation of ulcers/erosions in the glandular mucosa of the stomach of a single rabbit in the 500 mg/kg bw/day group was considered to possibly be related to treatment. There were no treatment-related maternal findings observed in the 25, 100 or 250 mg/kg bw/day groups. **The maternal LOAEL is 500 mg/kg bw/day based on clinical signs and body weight changes. The NOAEL is 250 mg/kg bw/day.** There were no treatment-related developmental nor teratogenic effects noted at any dose level tested. Hence, **the developmental LOAEL could not be determined. The developmental NOAEL is 500 mg/kg bw/day.**

The developmental toxicity study in the rabbit is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-143; TSN102319
Purity:	94.5% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** 0.5% Methocel A4M

3 Test Animals:

Species:	Rabbits, time-mated females.	
Strain:	New Zealand White	
Age/weight at study initiation:	5 - 6 months of age; 2500 - 3500 g.	
Source:	Covance Research Products, Inc., Kalamazoo, Michigan.	
Housing:	Individually in suspended stainless steel cages with flattened tube grid floors.	
Diet:	LabDiet Certified Rabbit Diet #5325, in pelleted form, *ad libitum*	
Water:	Municiple water, *ad libitum*	
Environmental conditions:	**Temperature:**	20±3°C
	Humidity:	40-60%
	Air changes:	12-15/hr
	Photoperiod:	12hrs dark/12 hrs light
Acclimation period:	~6 days.	

B. PROCEDURES AND STUDY DESIGN

1. **In Life Dates:** Phase I: April 9, 2001 to May, 2001;
Phase II: September 25, 2001 to October 25, 2001.

2. **Mating:** Sexually mature, virgin female rabbits were naturally mated with males of the same strain at Covance Research Products, Inc. The observed day of breeding was considered day 0 of gestation. Rabbits were shipped on day 0 or day 1 of gestation and arrived at the performing laboratory on the same day.

3. **Animal Assignment:** Animals were randomly assigned to dose groups as indicated in Tables 1a and 1b using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of the study.

TABLE 1a - Animal Assignment, Phase I

Dose (mg/kg bw/day)	0	25	100	250
# Females	26	26	26	26

TABLE 1b - Animal Assignment, Phase II

Dose (mg/kg bw/day)	0	500	750
# Females	26	26	26

4. Dose Selection Rationale: The dose levels for Phase I were selected based on the preliminary results of the probe study (MRID 46235634). The 250 mg/kg bw/day dose was expected to induce overt signs of maternal toxicity, i.e., decreased body weight. The lower dose levels were selected to provide dose response data for any toxicity that may be observed among the high-dose rabbits. However, rabbits at 250 mg/kg bw/day in the full study did not exhibit the significant body weight gain reductions that were observed at 250 mg/kg bw/day in the probe study. Consequently, a second dosing phase was added to this study. Dose levels for Phase II were chosen based upon body weight gain decreases and decreased food consumption observed at doses of 500 and 750 mg/kg bw/day in the probe study discussed previously.

5. Dosage Preparation and Analysis: Dosing suspensions were freshly prepared periodically (further details not provided) during the dosing period. The appropriate amount of test material was suspended in an aqueous solution of 0.5% Methocel A4M.
During the course of the study, stability of the test substance in suspension was evaluated for a period of 14 days at 25 and 250 mg/kg bw/day. Homogeneity was evaluated prior to initiation of Phases I and II at 25 and 250 mg/kg bw/day, and 500 and 750 mg/kg bw/day, respectively. Samples of test material suspensions at all dose levels were taken from the first preparations for both Phase I and Phase II and were analyzed for actual test material concentration.

Results:

Stability Analysis: The actual concentration of XDE-750 in the 25 and 250 mg/kg bw/day formulations, after 14 days storage, expressed as percentage of the initial concentration, were 106.4% and 90.1%, respectively.

Homogeneity Analysis: For Phase I, individual samples of the 25 and 250 mg/kg bw/day suspensions ranged from 99.2% to 105.8%, and 102.1% to 110.1% of the nominal concentrations, respectively. For Phase II, individual samples of the 500 and 750 mg/kg bw/day suspensions ranged from 100.8% to 103.2%, and 100.3% to 110.3% of the nominal concentrations, respectively.

Concentration Analysis: Individual samples of the 25, 100 and 250 mg/kg bw test suspensions from Phase I, and of the 500 and 750 mg/kg bw/day solutions from Phase II ranged from 102% to 106%, of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance

between nominal and actual dosage to the study animals was acceptable.

6. **Dosage Administration:** All doses were administered once daily by oral gavage, on gestation days 7 through 27, at a dose volume of 4 mL/kg bw/day. Dose volumes were adjusted daily based on individual body weights.

C. OBSERVATIONS

1. **Maternal Observations and Evaluations:** Clinical examinations were conducted daily throughout the study period, which included hand-held evaluation of the skin, fur, mucous membranes, respiration, nervous system function, unusual swelling or masses and animal behavior. In addition, twice daily cage-side examinations were conducted, at which time the skin, fur, mucous membranes, respiration, nervous system function, behavior, mortality, moribundity and availability of food and water were evaluated. Body weights were measured on day 0, day 4, daily during the dosing period and on day 28. Food consumption was recorded on a daily basis beginning on day 4. Dams were sacrificed on day 28 by IV injection of Beuthanasia-D Special, and were then necropsied. At that time, the uterus, liver and kidneys were removed and weighed. The uterus was examined for the number of implantation sites, number and location of live and dead fetuses, and number, location and classification of resorption sites. Resorption sites were classified as either early or late based on the presence (late resorption) or absence (early resorption) of grossly recognizable embryonic/fetal form. The ovaries were examined for the number of corpora lutea. Uteri which appeared non-pregnant were stained with a 10% aqueous solution of sodium sulfide and examined for evidence of early resorptions to determine pregnancy status. Representative samples of liver with gallbladder, kidneys and gross lesions were preserved in neutral, buffered 10% formalin. Microscopic examination of tissues was not conducted.
Any animal that died, appeared moribund or showed indications of premature delivery was submitted for a complete necropsy by a veterinary pathologist. However, the liver, kidney and gravid uterine weights were not recorded, nor were the number of corpora lutea and the sex and body weight of fetuses from these animals recorded. Development of the conceptuses was determined to the extent possible by external examination (as appropriate for gestational age). The conceptuses were not examined for visceral or skeletal alterations. Following external examination, these conceptuses were discarded. Near term fetuses were euthanized by oral administration of sodium pentobarbital solution.

2. **Fetal Evaluations:** All fetuses were removed from the uterus, weighed and sexed, and then examined for external abnormalities. Fetuses were euthanized by oral administration of sodium pentobarbital solution. All fetuses were examined by dissection under a low power stereomicroscope for visceral abnormalities. The head from at least one-half of the fetuses were immersed in Bouin's fluid, then serially sectioned for examination of the eyes, brain, nasal passages and tongue. All fetuses were preserved in alcohol, cleared, stained with Alizarin red-S and then examined for skeletal alterations.

3. **Classification of Fetal Findings:**
a) Malformations: A permanent structural change that may adversely affect survival, development or function and/or which occurs at a relatively low incidence in the specific species/strain.
b) Variations: A divergence beyond the normal range of structural constitution that may not adversely affect survival or health.

D. DATA ANALYSIS

1. **Statistical Analyses:** Maternal body weights, maternal body weight gains, organ weights (absolute and

relative), fetal body weights and feed consumption were evaluated by Bartletts test (alpha=0.01) for equality of variances. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha=0.05, analysis by Dunnett's test (alpha=0.05) or the Wilcoxon Rank-Sum test (alpha=0.05) with Bonferroni's correction was performed, respectively. Frequency of pre-implantation loss, post-implantation loss (calculations shown below), resorptions per litter and resorptions per fetal population and fetal alterations were analyzed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implantations, and litter size was evaluated using a nonparametric ANOVA (alpha=0.05) followed by the Wilcoxon Rank-Sum test (alpha=0.05) with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test (alpha=0.05) with Bonferroni's correction. Fetal sex ratios were analyzed using a binomial distribution test. Non-pregnant females, females with resorptions only, or females found to be pregnant after staining of their uteri were excluded from the appropriate analyses. Statistical outliers were identified, using a sequential method (alpha=0.02), and excluded if justified by sound scientific reasons. Both Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control group to keep the experiment-wise alpha at 0.05. Both were reported at the experiment-wise alpha level. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data took into consideration statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

2. Indices: The following indices were calculated from caesarean section records of animals in the study:
a) Pre-implantation loss* = (# corpora lutea - implantations) x 100/# corpora lutea
b) Post-implantation loss* = (# implantations - live born pups) x 100/# implantations

* Note: Percent pre- and post-implantation losses were determined for each litter, followed by calculation of the mean of these litter values.

3. Historical Control Data: Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality: a) Phase I: Findings were as follows:
i) Control group: No deaths.
ii) 25 mg/kg bw/day group: One female aborted 4 fetuses on study day 26, and so was euthanized. Gross examination of the uterine contents revealed 7 fetuses, which appeared normal, and 1 late resorption still within the uterus. The only other findings were perineal soiling and dark ingesta/watery contents of the cecum. The cause of abortion was undetermined.
iii) 100 mg/kg bw/day group: No deaths.
iv) 250 mg/kg bw/day group: One female was removed from the study on day 3, prior to initiation of treatment, due to anorexia, decreased appetite and malodorous, soft, watery feces.
A second female aborted one resorbing fetus on study day 19, and so was euthanized. Gross examination revealed pale kidneys and evidence of recent abortion. The cause of abortion was undetermined.
A third female was found dead on study day 15. This rabbit exhibited reflux of test material at dosing with subsequent noisy and labored respiration. Necropsy confirmed gavage error, i.e., mottled lungs with edema, froth in the trachea and bronchi and blood tinged froth on the nares and muzzle. Examination of

the uterus revealed 14 fetuses, which appeared normal upon gross examination, and 1 early resorption.

The above-noted findings were not considered to be treatment-related.

B) Phase II: Findings were as follows:
i) Control group: No deaths.
ii) 500 mg/kg bw/day group: One female was found dead on study day 27. Gross necropsy revealed dark consolidation in both lungs with accumulation of serosanguinous fluid and abnormal lung distension, froth in the trachea and bronchi and frothy red discharge around the nares and muzzle. It was stated that this dam was pregnant, but further information on the fetuses was not available. It was concluded that this death was due to gavage error.
iii) 750 mg/kg bw/day group: Two dams were sacrificed moribund on study day 17. Clinical findings were incoordinated gait, perineal urine soiling, decreased feces, decreased activity and significant decreases in food intake and body weight gain. Gross examination revealed pale kidneys, watery/dark cecal contents, erosions/ulcers in the glandular mucosa of the stomach and hairballs. Both dams were pregnant; information on the fetuses was not provided.
One female was found dead on study day 8. Post mortem examination revealed perineal soiling, congestion in the kidneys and liver, watery contents in the cecum, lung consolidation, abnormal lung distension, exudate in the bronchus, mucoid exudate and froth in the trachea and petechia in the stomach. Examination of the uterus revealed 14 implantations including 4 resorptions. It was reported that the fetuses were normal. It was concluded that this death was due to gavage error.

The moribund condition of the 2 dams in the 750 mg/kg bw/day group was considered to be treatment-related.

2. Clinical Observations: a) Phase I: There were no overt, clinical signs of treatment-related toxicity.
b) Phase II: In the 500 and 750 mg/kg bw/day groups, there was an increased incidence of incoordinated gait, i.e., 23/26 dams were affected in each of the 500 and 750 mg/kg bw/day groups, versus 0/26 dams in the control group. The incoordination observed at 750 mg/kg bw/day was characterized by a reluctance to move unless manually stimulated by the observer. Significant stiffening or dragging of the limbs was evident when movement was attempted and on various occasions several rabbits tipped onto their sides. In most cases, the incoordination was transient, with complete resolution within two hours postdosing, and did not appear to progressively worsen on subsequent days. In the 500 mg/kg bw/day dose group, the degree of incoordination observed was less pronounced than that observed in the 750 mg/kg bw/day rabbits. In general, when stimulated to move, the 500 mg/kg bw/day rabbits exhibited cautious placement of their paws with a slight stiffening or dragging of the forelimbs, but none tipped over when moving. The incoordination observed at 500 mg/kg bw/day resolved within two hours of dosing. The only other clinical observation was decreased amounts of feces observed in the 750 mg/kg bw/day group.
Due to the severity of clinical signs and corresponding significant decreases in body weight gain (see below) on gestation days 7-10 observed in the majority of the 750 mg/kg bw/day rabbits, this entire dose group was removed from study on October 8, 2001 (gd 11-20).

2. Body Weight: a) Phase I: Refer to Table 2a. There were no treatment-related effects on body weight or body weight gain at any dose level tested.

TABLE 2a - Phase I - Maternal Body Weight and Body Weight Gain, (g)±SD[a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (26)	25 (24/23)	100 (25)	250 (22/21)
Initial Body Weight	3241.5±178.3	3125.6±215.8	3181.2±170.2	3231.1±190.8
Pre-treatment (gain):				
Days - 0 to 4	24.2±85.4	63.8±78.6	24.8±61.3	46.9±97.3
4 to 7	83.1±69.9	79.4±73.2	93.5±76.0	70.0±66.6
Treatment (gain):				
Days - 7 to 28	299.2±124.8	271.3±150.2	319.3±119.1	250.8±138.1
Body weight gain, d. 0 to 28	406.5±188.9	415.7±167.4	437.6±134.1	364.8±162.6
Final body weight	3183.0±244.2	3084.5±241.4	3154.8±168.5	3105.9±179.5
Gravid Uterus Weight	464.94±3.86	444.72±104.53	464.05±102.13	478.88±86.61

[a] Data extracted from pages 42 to 45 and 69 of the study report

b) Phase II: Refer to Table 2b. In the 500 mg/kg bw/day group, the only treatment-related finding was a net loss in body weight during the first 3 days of treatment only. However, during subsequent time intervals, body weight gain was comparable to or higher than the control group values. In addition, body weights throughout the study and overall body weight gain in the 500 mg/kg bw/day group were comparable to the control group values. In the 750 mg/kg bw/day group, a net loss in body weight was seen during the first 3 days of treatment, and body weight gain was slightly lower during days 13 to 16. The study authors stated that "due to the magnitude of the body weight gain decrease seen on gestation days 7-10 and the severity of the clinical signs observed in several 750 mg/kg bw/day rabbits, this entire dose group was removed from the study early without further investigation."

TABLE 2b - Phase II - Maternal Body Weight and Body Weight Gain, (g)±SD[a]

Interval	Dose in mg/kg bw/day (# of Dams)		
	0 (25)	500 (25)	750[b]
Initial Body Weight	3162.9±183.3	3186.5±232.8	3089.4±229.4 (26)
Pre-treatment (gain):			
Days - 0 to 4	27.0±78.0	23.8±60.8	19.0±68.7 (26)
4 to 7	50.1±61.2	48.2±54.1	35.3±60.3 (26)
Treatment (gain):			
Days - 7 to 10	25.0±37.7	-11.8±54.3*	-70.0±111.3* (25)
10 to 13	39.5±53.3	51.9±68.8	63.5±104.2 (15)
13 to 16	97.3±41.6	91.8±51.3	33.2±104.6 (12)
16 to 20	36.8±63.7	50.4±60.6	---
20 to 24	45.0±43.4	55.4±46.3	---
24 to 28	39.1±68.4	57.8±58.5	---
7 to 28	282.6±124.9	305.9±93.9	---
Body weight, day 7	3240.0±224.2	3258.5±240.7	3143.7±208.9
day 10	3265.0±227.9	3246.7±263.9	3068.7±205.9*
day 13	3304.4±228.6	3298.6±245.3	3184.5±229.1
day 16	3401.7±243.8	3390.4±263.1	3206.3±311.2
Body weight gain, d. 0 to 28	359.7±152.3	374.5±117.9	---

Final body weight	3096.1±221.4	3105.7±251.5	---
Gravid Uterus Weight	426.49±118.06	461.97±77.62	NA

[a] Data extracted from pages 42 to 45 and 70 of the study report
[b] Varying N values due to death of one dam and early termination of the entire dose group (animals were at various stages of gestation).

3. Food Consumption: a) Phase I: There was no treatment-related effect on food intake at any dose level tested.
Daily mean food intake values (percent of control group value in brackets) for the 0, 25, 100 and 250 mg/kg bw/day groups, respectively, were as follows:
164.4 g; 158.7 g (96.5%); 170.1 g (103.4%); and 155.1 g (94.3%).
b) Phase II: Overall mean food intake was lower in the 750 mg/kg bw/day group due to lower daily intake to day 20 (i.e., study termination for the 750 mg/kg bw/day group). Although food intake was lower in the 500 mg/kg bw/day group during the first 3 days of treatment, i.e., days 7 - 10, attaining statistical significance for days 7 - 8, overall food intake was only slightly lower than the control group value.
Daily mean food intake values to day 20 (percent of control group value in brackets) for the 0, 500 and 750 mg/kg bw/day groups, respectively, were as follows:
170.5 g; 158.7 g (93.1%); and 130.0 g (76.3%).
[Daily mean food intake values to day 28 (percent of control group value in brackets) for the 0 and 500 mg/kg bw/day groups, respectively, were 161.3 g; and 153.0 g (94.8%).]

4. Organ Weights: Phase and Phase II: There were no treatment-related findings in the 25, 100, 250 or 500 mg/kg bw/day groups. (Organ weights were not evaluated in the 750 mg/kg bw/day group).

5. Gross Pathology: a) Phase I: There were no treatment-related findings.
b) Phase II: Refer to Table 3. In the 500 mg/kg bw/day group, the only finding considered to possibly be related to treatment was the observation of ulcers/erosions in the glandular mucosa of the stomach of a single rabbit.
In the 750 mg/kg bw/day group, there was an increased incidence of pale kidneys and of ulcers/erosions in the glandular mucosa of the stomach.

TABLE 3 - Selected Gross Pathological Findings[a]

Observations	Dose (mg/kg bw/day)		
	0	500	7500
Kidneys, pale	38011	38011	38316
Stomach, erosions/ulcers	38011	38011	38255

[a] Data extracted from pages 52 to 68, and pages 453 to 504 of the study report.

5. Caesarean Section Data: a) Phase I: Refer to Table 4a. There were no treatment-related findings.

TABLE 4a - Caesarean Section Observations[a]

Observation	Dose (mg/kg bw/day)			
	0	25	100	250
# Animals Assigned (Mated)	26	26	26	26
# Animals Pregnant Pregnancy Rate (%)	26/26 100	24/26 92.3	25/26 96.2	23/25[b] 92.0
# Aborted	0	1	0	1[c]
Gravid Uterus Weight, g	464.94±3.86	444.72±104.53	464.05±102.13	478.88±86.61
# with all dead/resorbed	0	0	0	1
# Corpora Lutea/Dam	10.1±1.98.5	10.1±2.0	9.7±2.4	9.7±1.9
# Implantations/Dam	8.8±2.0	8.3±2.3	8.6±2.5	8.9±2.0
Total # Litters	26	23	25	21
# Live Fetuses/Dam	8.5±2.0	8.1±2.2	8.4±2.3	8.6±1.7
Mean Fetal Weight (g): males females	37.11±3.80 34.80±4.49	37.17±3.17 35.86±3.23	37.38±4.51 36.94±4.96	36.56±3.59 35.56±4.11
Sex Ratio (% Male)	53	46	49	48
Resorptions/Litter	0.3±0.5	0.3±0.4	0.2±0.4	0.3±0.6
Preimplantation loss (%)	12.0±15.6	18.5±15.6*	11.6±16.9	8.5±12.2
Postimplantation Loss (%)	4.2±6.4	2.8±5.0	1.9±3.9	2.7±5.9

[a] Data extracted from page 69 of the study report.
[b] One female was removed from study on gestation day 3; pregnancy status not determined.
[c] One female aborted one resorbed fetus.
* Statistically significantly different from control, $p<0.05$

b) **Phase II:** Refer to Table 4b. There were no treatment-related findings.

TABLE 4b - Caesarean Section Observations[a]

Observation	Dose (mg/kg bw/day)		
	0	500	750
# Animals Assigned (Mated)	26	26	26
# Animals Pregnant Pregnancy Rate (%)	25/26 96.2	25/26 96.2	26/26 100
# Aborted	0	0	0
Gravid Uterus Weight, g	426.49±118.06	461.97±77.62	---
# with all dead/resorbed	0	0	---
# Corpora Lutea/Dam	10.0±2.1	10.6±2.4	---
# Implantations/Dam	8.0±2.4	9.0±2.2	---
Total # Litters	25	24	---

# Live Fetuses/Dam	7.7±2.4	8.8±2.0	---
Mean Fetal Weight (g): males females	36.81±4.54 35.76±4.17	35.41±3.75 34.95±3.55	--- ---
Sex Ratio (% Male)	48	52	---
Resorptions/Litter	0.3±0.5	0.3±0.4	---
Preimplantation loss (%)	20.3±22.6	14.5±12.9	---
Postimplantation Loss (%)	3.5±7.1	2.3±4.1	---

[a] Data extracted from page 70 of the study report.

B. DEVELOPMENTAL TOXICITY

1. External Examination: Phase I and Phase II: There were no external malformations or variations noted at any dose level tested.

2. Visceral Examination: a) Phase I: Refer to Table 4a. There was no evidence of any treatment-related effects on the incidence of visceral malformations or variations at any dose level of XDE-750 tested. The specific malformations noted were as follows:

i) At 0 mg/kg bw/day: bifurcated renal artery.
ii) At 25 mg/kg bw/day: missing gall bladder; bifurcated renal artery (3 individual fetuses).
iii) At 100 mg/kg bw/day: missing gall bladder; missing gall bladder, diaphragmatic hernia and missing lung lobes - multiple; persistent truncus arteriosis aorta; and bifurcated renal artery.
iv) At 250 mg/kg bw/day: displaced aortic arch and missing innominate artery; persistent truncus arteriosis aorta; and bifurcated renal artery.

TABLE 4a - Visceral Examinations - Litter (fetal) incidence[a]

Observations	Dose (mg/kg bw/day)			
	0	25	100	250
#Litters (fetuses) examined	26 (221)	23 (186)	25 (209)	21 (180)
Total Malformations Total Variations	1 (1) 9 (24)	3 (4) 10 (23)	4 (4) 10 (20)	3 (3) 7 (13)

[a] Data extracted from pages 71 to 73, and pages 511 to 651 of the study report.

b) Phase II: Refer to Table 4b. There was no evidence of any treatment-related effects on the incidence of visceral malformations or variations at any dose level of XDE-750 tested. The specific malformations noted were as follows:

i) At 0 mg/kg bw/day: misshapen ventricle, misshapen semilunar valve, septal defect ventricle and transposition of great vessels; bifurcated renal artery (6 individual fetuses); dilated cerebral ventricles.
ii) At 500 mg/kg bw/day: missing testis.

TABLE 4b - Visceral Examinations - Litter (fetal) incidence[a]

Observations	Dose (mg/kg bw/day)		
	0	500	750
#Litters (fetuses) examined	25 (192)	24 (210)	---
Total Malformations	5 (8)	1 (1)	---
Total Variations	12 (15)	12 (22)	---

[a]Data extracted from pages 78 to 80, and pages 652 to 721 of the study report.

3. Skeletal Examination: **a) Phase I:** Refer to Table 5a. There was no evidence of any treatment-related effects on the incidence of skeletal malformations or variations at any dose level of XDE-750 tested. The specific malformations noted were as follows:

i) At 0 mg/kg bw/day: fused thoracic vertebrae, hemivertebrae thoracic vertebrae and fused thoracic centra.
ii) At 25 mg/kg bw/day: no malformations.
iii) At 100 mg/kg bw/day: hemivertebrae atlas (2 individual fetuses); hemivertebrae thoracic vertebrae, forked ribs and fused thoracic rib; hemivertebrae thoracic vertebrae, fused thoracic centra and fused thoracic rib; Class II wavy thoracic rib; and Class II wavy thoracic rib, misshapen scapula, misshapen humerus, misshapen radius, misshapen ulna, misshapen femur, misshapen fibula and misshapen tibia.
iv) At 250 mg/kg bw/day: no malformations.

TABLE 5a - Skeletal Examinations - Litter (fetal) incidence[a]

Observations	Dose (mg/kg bw/day)			
	0	25	100	250
#Litters (fetuses) examined:				
Head	26 (104)	23 (87)	25 (99)	21 (86)
Body	26 (221)	23 (186)	25 (209)	21 (180)
Total Malformations:				
Head	0 (0)	0 (0)	1 (2)	0 (0)
Body	1 (1)	0 (0)	3 (3)	0 (0)
Total Variations:				
Head and Body	23 (90)	19 (70)	21 (86)	18 (71)

[a]Data extracted from pages 71 to 73, and pages 511 to 651 of the study report.

a) Phase II: Refer to Table 5b. There was no evidence of any treatment-related effects on the incidence of skeletal malformations or variations at any dose level of XDE-750 tested. The specific malformations noted were as follows:

i) At 0 mg/kg bw/day: missing cervical vertebrae; and fused thoracic rib (2 individual fetuses).
ii) At 500 mg/kg bw/day: fused thoracic centra; and hemivertebrae thoracic vertebrae and fused thoracic centra.

TABLE 5b - Skeletal Examinations - Litter (fetal) incidence[a]

Observations	Dose (mg/kg bw/day)		
	0	500	750
#Litters (fetuses) examined:			
Head	24 (88)	24 (100)	---
Body	25 (192)	24 (210)	---
Total Malformations:			
Head	1 (1)	0 (0)	---
Body	2 (2)	1 (2)	---
Total Variations:			
Head and Body	21 (76)	24 (110)	---

[a]Data extracted from pages 78 to 80, and pages 652 to 721 of the study report.

III. DISCUSSION

A. Investigators' Conclusions: "Phase I rabbits given ≤ 250 mg/kg/day XDE-750 did not exhibit any treatment-related maternal or developmental toxicity, thus prompting the evaluation of higher doses in Phase II. At 500 mg/kg/day, maternal toxicity was evidenced by transient incoordinated gait (23/26 does) and decreased body weight gains (gd 7-10), but there was no evidence of embryonal/fetal effects. At 750 mg/kg/day, incoordinated gait was accompanied by decreased amounts of feces, significant reductions in body weight, body weight losses, decreased body weight gains, and decreased feed consumption. Also, two 750 mg/kg/day does were euthanized in moribund condition (gd 17). At necropsy, these does had pale kidneys, watery, dark cecal contents, erosions/ulcers of the stomach (glandular mucosa) and hairballs. As 750 mg/kg/day exceeded the maximum tolerated dose, all surviving rabbits in the dose group were removed from study. Based upon these results, the no-observed-effect-levels (NOEL) for maternal and developmental toxicity were 250 mg/kg/day and 500 mg/kg/day, respectively."

B. Reviewer's Discussion:

1. Maternal Toxicity: New Zealand White rabbits were dosed by oral intubation with XDE-750 technical, purity 94.5%, as a suspension in 0.5% Methocel A4M, at dose levels of 0, 25, 100 or 250 mg/kg bw/day (Phase I) or 0 , 500 or 750 mg/kg bw/day (Phase II), 26 females per group, from day 7 to 27 of gestation, inclusive. Two animals in the 750 mg/kg bw/day group were sacrificed in a moribund condition (incoordinated gait, significant body weight losses, decreased food intake), which was considered to be treatment-related. In the 500 and 750 mg/kg bw/day groups, there was an increased incidence of incoordinated gait, which was more pronounced in the 750 mg/kg bw/day group. In most cases, the incoordination was transient, with complete resolution within two hours post-dosing, and did not appear to progressively worsen on subsequent days. The only other clinical observation was decreased amounts of feces observed in the 750 mg/kg bw/day group. There was a net loss in body weight in the 500 and 750 mg/kg bw/day groups during the first 3 days of the dosing period (days 7 to 10), with a corresponding decrease in food consumption, which was more pronounced in the 750 mg/kg bw/day group. The 750 mg/kg bw/day group was removed from the study by day 20 due to the severity of clinical signs and decreases in body weight gain on gestation days 7-10. At necropsy, there was an increased incidence of pale kidneys and of ulcers/erosions in the glandular mucosa of the stomach in the 750 mg/kg bw/day group. The observation of ulcers/erosions in the glandular mucosa of the stomach of a single rabbit in the 500 mg/kg bw/day group was considered to possibly be related to treatment.

Maternal NOAEL = 250 mg/kg bw/day.
Maternal LOAEL = 500 mg/kg bw/day.

2. Developmental Toxicity:

a. Deaths/Resorptions: The number of resorptions/dam and viable fetuses/dam were comparable between the concurrent control and treatment groups.

b. Altered Growth: There was no treatment-related effect on mean fetal body weights.

c. Developmental Variations: There were no treatment-related developmental variations noted at any dose level tested.

d. Malformations: There were no treatment-related developmental malformations noted at any dose level tested.

Developmental NOAEL = 500 mg/kg bw/day
Developmental LOAEL could not be determined since there were no adverse, treatment-related findings at any dose level tested.

C. Study Deficiencies: There were no scientific deficiencies noted in the study.

005100
(15)

Aminopyralid Liquid Concentrate / DOW aminopyralid [AMD] / PMRA Sub. No. 2004-0790 ~ PROTECTED ~ Rat developmental toxicity / 1 DACO 4.5.2 / OECD IIA 5.6.2.1

PMRA Primary Reviewer: Steve Wong, Ph.D. **Signature:**
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509 **Date** 9/16/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Prenatal developmental study - Rat; OPPTS 870.3700; OECD 414.

PC CODE: 005100 005209 **DP BARCODE**: D305670

TEST MATERIAL (PURITY): GF-871 (Test material is 41.3% XDE-750 TIPA salt in water)

SYNONYMS:

XDE-750 triisopropanolammonium, XDE-750 TIPA, aminopyralid triisopropanolammonium, X677349

CITATION: Bjorn, AT, 25 September 2003. GF-871: An oral developmental toxicity study in Sprague Dawley rats. Charles River Laboratories, Inc. Discovery and Development Services, Spencerville, OH. Dow Study ID 031141, Charles River Study ID 3504.344. Unpublished

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 46235631), in Sprague Dawley (Crj:CD®(SD)IGS BR) rats, GF-871 (containing 41.5% XDE-750 triisopropanolammonium, TIPA) in deionized water was administered by oral gavage to groups of 25 mated females at dose levels of 0, 484, 1211, or 2421 mg/kg bw/d (or 0, 200, 500, or 1000 mg/kg bw/d in terms of XDE-750 TIPA) at a constant volume of 4 mL/kg bw on gestation days 6-19. The acid equivalent (ae) doses are 0, 104, 260, and 520 mg ae/kg bw/d. The animals were sacrificed on gestation day 20. There were no deaths. There were no toxicologically meaningful clinical observations in any animals. A few clinical signs were observed sporadically throughout the groups; however, the findings were minor and did not follow a consistent pattern or dose response that would indicate a relationship to treatment. There were no statistically significant or toxicologically meaningful differences in mean body weights, body weight changes, mean maternal body weight change or food consumption between the control and test groups. Gross necropsy findings at scheduled necropsy were generally unremarkable and there were no statistically significant or toxicologically meaningful differences in absolute liver and kidney weights or organ-to-body weight ratios between the control and test animals. There were no toxicologically meaningful differences in cesarean section parameters between the control and test groups. No statistically significant or toxicologically meaningful differences were noted among the groups with respect to fetal malformations or developmental variations.

The maternal and developmental LOAEL was not determined. The NOAEL for maternal and developmental toxicity was 2421 mg/kg bw/d (or 1000 mg/kg bw/d based on XDE-750 TIPA). The acid equivalent dose is 520 mg ae/kg bw/d. There was no evidence of teratogenicity.

The developmental toxicity study in the rabbit is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was performed in accordance to the US EPA, Health Effects Test Guidelines, OPPTS 870.3700, 1998; OECD Guideline No. 414, Prenatal Developmental Toxicity Study, 2001; EEC Methods, Teratology Study, Methods for the Determination of Toxicity, Official Journal of the European Communities, Vol. 31, No L 133, 30 May 1998; and JMAFF Notification of 12 NohSan-8147, Guideline 2-1-18, Teratology Study, 24 November 2000.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test material:** GF-871

Description:	brown liquid; stored at room temperature in sealed containers
Lot/Batch #:	173-162-1A
Purity:	Contains 41.3% XDE-750 TIPA salt as ai
Compound stability:	"Stability of the test article in the vehicle was evaluated at 15 and 24 days post-preparation (storage at room temperature)."
CAS #:	150114-71-9 and 000122-20-3

2. **Vehicle control:** deionized water

3 **Test animals:**

Species:	rat	
Strain:	Sprague Dawley Crj:CD®(SD)IGS BR	
Age/weight at study initiation:	11 weeks; 200-250 g	
Source:	Charles River Labs Inc, Raleigh, NC	
Housing:	individually in suspended stainless steel cages	
Diet:	PMI Certified Rodent Chow #5002 (PMI Nutrition International) *ad libitum*	
Water:	municipal tap water *ad libitum*	
Environmental conditions:	**Temperature:**	19-23 °C
	Humidity:	43-54 %
	Air changes:	10-15 exchanges/h
	Photoperiod:	12 h dark / 12 h light
Acclimation period:	no information	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - Start: September 25, 2003 End: October 17, 2003

2. **Mating:**

Female rats were mated over three days with breeder male rats (1:1 ratio) at the supplier's animal facility (Charles River Laboratories, Inc., Raleigh, North Carolina). The day evidence of copulation was confirmed was designated as gestation day 0 and resulted in separation of the mating pair.

3. **Animal assignment:**

The females were randomly assigned by a computer generated program to the test and control groups as indicated in Table 1.

Table 1: Animal assignment

Dose, mg GF-871/kg bw/d	0	484	1211	2421
Dose, mg XDE-750 TIPA/kg bw/d	0	200	500	1000
Number of mated ♀	25	25	25	25

4. **Dose selection rationale:**

The dose levels were chosen in an attempt to produce graded responses to the test article.

5. **Dosage preparation and analysis**

For each dosage group, an appropriate amount of GF-871 was weighed into a pre-calibrated beaker. Deionized water, 50 mL, was added and the solution was hand stirred. The desired concentration was adjusted by adding more deionized water. Each dosing solution was stirred using a magnetic stir bar for ≥15 minutes, then was dispensed into individual amber containers for daily dosing. The dosing preparations were prepared twice during the study for up to 9 days of dosing. The dosing preparations were stored at room temperature prior to administration to test animals. Dosing solutions were corrected for the concentration (41.3%) of the active ingredient in the GF-871 formulation. Throughout this report, dose levels are expressed in terms of the active ingredient, XDE-750 TIPA.

Concentrations of the test article, which encompassed the expected low- and high-dose levels for the study, were evaluated. Stability of the test article in the vehicle was evaluated at 15 and 24 days post-preparation (storage at room temperature).

Results:

Stability analysis:
Aqueous solutions of GF-871 at concentrations of 50 and 250 mg/mL were stable for at least 22 days. The concentrations after storage were 96.0 to 102 % of the initial concentrations.

Concentration analysis:
Analyses of all dosing solutions revealed concentrations of 97.2 to 99.2 % of the target levels.

Stability and concentration analysis data indicated that the mixing procedures and dosing preparations were adequate and acceptable.

6. **Dosage administration:**

All doses were administered once daily by gavage, on gestation days 6 through 19, in a volume of 4 mL/kg bw/d. Dosing was based on the body weight on the most recent body weight determination.

C. **OBSERVATIONS**

1. **Maternal observations and evaluations** -

The animals were observed daily for mortality and clinical toxic signs. Individual body weight and food consumption were recorded on gestation days 0 (body weight only), 5, 6, 9, 12, 15, 18 and 20. On gestation day 20, all survivors were sacrificed by CO_2 anaesthesia and examined for gross pathologic changes. The weights of the liver and kidneys were recorded. The uterus was removed from the body, weighed intact (gravid uterus only), examined externally, and then opened for internal examination. The number of viable and non-viable fetuses and early and late resorptions were recorded. Placentas

were examined and the number of corpora lutea on each ovary was recorded. Uteri with no evidence of implants were opened and placed in 10% aqueous ammonium sulfide solution for detection of early embryo lethality.

2. **Fetal evaluations**:

Fetuses were examined for external, visceral, and skeletal abnormalities. Findings were classified, using nomenclature accepted in developmental toxicology evaluations, as malformations or developmental variations based on the severity of the anatomical change(s) and their potential for interference with normal organ and/or body functions. Each fetus was examined externally, sexed, weighed. Approximately one-half of the fetuses from each litter were fixed in Bouin's solution for subsequent visceral examination using Wilson's technique. Approximately one-half of the fetuses from each litter were fixed in 95% isopropyl alcohol. Following fixation, the fetuses were macerated in a 1-2% aqueous potassium hydroxide solution, stained with Alizarin Red S and cleared in glycerin. The skeletal examination was performed using a low power microscope.

D. DATA ANALYSIS

1. **Statistical analyses**:

Maternal survival, total implant loss, viable fetuses, and fetal malformation and variation data were compared among the groups by R x C Chi-square test followed by Fisher's Exact Test for group-wise comparisons to the control group, when appropriate. Body weights, body weight changes, food consumption, maternal liver and kidney weights, gravid uterine weights, corpora lutea counts, number of implantation sites, number of live fetuses and fetal body weights were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test for group-wise comparisons to the control group, when appropriate. In addition to mean and standard deviation (SD) calculations, group mean percentages were calculated for the following cesarean section parameters: pre-implantation loss, fetal sex distributions, post-implantation loss, dead fetuses, and early and late resorptions. The percentage data, number of dead fetuses, and number of early and late resorptions were analyzed by Kruskal-Wallis non-parametric ANOVA. When employed, Kruskal-Wallis was used for both the multiple group comparisons and group-wise comparisons to the control group, as appropriate. All calculations and statistical analyses were based on the female or litter as the experimental unit. Statistical significance was reported at an alpha level of $p<0.05$. Data from non-pregnant rats were excluded from statistical analysis.

2. **Historical control data**:

Historical control data were provided to allow comparison with concurrent controls. Historical control data were not necessary due to the absence of treatment-related systemic toxicity or developmental effects.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations**:

There were no mortality or compound-related changes in the appearance and behavior of rats.

2. **Body weight** - Body weights were not affected.

3. **Food consumption** - There were no treatment-related effects.

4. Gross pathology - There were no treatment-related gross pathological findings.

5. Cesarean section Data - Data are presented in Table 2.

Table 2 Cesarean section observations

Observation	mg/kg bw/d (based on XDE-750 TIPA)			
	0	200	500	1000
# Animals assigned (mated ♀)	25	25	25	15
# Animals pregnant	24	25	25	25
Pregnancy rate (%)	96	100	100	100
# Non-pregnant	1	0	0	0
Maternal wastage				
# Died	0	0	0	0
# Died pregnant	0	0	0	0
# Died nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature delivery	0	0	0	0
Corpora lutea/Dam	14.7±2.54	15.4±1.83	15.2±2.11	15.0±1.99
Implantations/Dam	13.4±2.72	14.5±1.36	13.9±1.22	14.0±1.99
Pre-implantation loss/Dam	1.3±1.60	0.9±1.17	1.3±1.91	1.0±1.58
Total # litters	24	25	25	25
Live fetuses/Dam	12.8±3.08	13.8±1.43	12.8±1.54	12.8±2.48
Post-implantation loss/Dam	0.7±0.92	0.7±0.80	1.2±1.37	1.2±1.31
Total # dead fetuses	0	0	0	0
Total # resorptions:				
early	0.7±0.92	0.6±0.81	1.1±1.39	1.2±1.31
late	0.0±0.00	0.0±0.20	0.0±0.20	0.0±0.00
Litters with total resorptions	0	0	0	0
Mean gravid uterus weight, g	79±18.8	83±8.9	78±9.3	77±14.2
Mean fetal weight (g), ♂ ♀	♂ = 4.2±0.23 ♀ = 3.9±0.23	♂ = 4.0±0.23 ♀ = 3.8±0.23	♂ = 4.1±0.20 ♀ = 3.9±0.19	♂ = 4.0±0.29 ♀ = 3.8±0.22
Sex ratio (♂:♀)	54.5:45.5	48.9:51.1	46.0:54.0	51.9:48.1
data taken from Tables 5, 10, pp 33, 46-48 of Report				

B. DEVELOPMENTAL TOXICITY

There was no evidence of treatment-related external, visceral, or skeletal abnormalities. Fetal malformations were limited to two fetuses with folded retina and one fetus with situs inversus in the control group; two fetuses with situs inversus in the 200 mg/kgbw/d group; one fetus with lumbar vertebral anomaly and costal cartilage anomaly in the 500 mg/kg bw/d group; and one fetus with multiple malformations (fetal edema, polydactyly, heart and/or great vessel anomaly), and one fetus with costal cartilage anomaly in the 1000 mg/kg bw/d group. The type and incidence of fetal developmental variations was comparable among the groups. The fetal findings are presented in Table 3.

TABLE 3. Fetal examination for external, visceral and skeletal abnormalities

Observations		mg/kg bw/d (based on XDE-750 TIPA)			
		0	200	500	1000
External malformations	fetuses examined	304	346	319	320
	polydactyly	0	0	0	1
	total fetuses/litter with malformations	0	38352	38352	37256
Visceral malformation	fetuses examined	152	175	159	159
	folded retina	2	0	0	0
	heart and/or great vessel anomaly	0	0	0	1
	sinus inversus	1	2	0	0
	total fetuses/litter with abnormalities	38412	38383	38352	38352
Skeletal malformations	fetuses examined	154	171	160	161
	lumbar vertebral anomaly	0	0	1	0
	costal cartilage anomaly	0	0	1	1
	total fetuses/litter with malformations	38352	38352	38352	38352
data taken from Table 11, pages 50-52 of Report					

III. DISCUSSION

A. Investigators' conclusions:

"All females survived to scheduled cesarean section on gestation day 20. There were no toxicologically meaningful clinical observations in any of the study animals. A few clinical signs were observed sporadically throughout the groups; however, the findings were minor and did not follow a consistent pattern or dose response that would indicate a relationship to treatment. There were no statistically significant or toxicologically meaningful differences in mean body weights, body weight changes, mean maternal body weight change or food consumption between the control and test article-treated groups. Gross necropsy findings at scheduled necropsy were generally unremarkable and there were no statistically significant or toxicologically meaningful differences in absolute liver and kidney weights or organ-to-body weight ratios between the control and test article-treated groups. There were no toxicologically meaningful differences in cesarean section parameters between the control and test article-treated groups. No statistically significant or toxicologically meaningful differences were noted among the groups with respect to fetal malformations or developmental variations.

In conclusion, a dosage level of 1000 mg/kg/day of GF-871 was considered a no observed- effect level (NOEL) for maternal effects and fetal developmental effects."

B. Reviewer's discussion:

This study was properly conducted and reported. The conclusions of the authors are acceptable. GF-871 were not teratogenic to rats. The NOAELs for maternal toxicity, developmental toxicity, and teratogenicity are 1000 mg/kg bw/d, the highest dose level tested.

C. Study deficiencies: There are no deficiencies.

005100
(6)

Aminopyralid Liquid Concentrate / DOW aminopyralid [AMD] / PMRA Sub. No. 2004-0790 ~ PROTECTED ~ Rabbit developmental toxicity / 1 DACO 4.5.3 / OECD IIA 5.6.2.2

PMRA Primary Reviewer: Steve Wong, Ph.D.
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: Steve Wong
Date Sept 29, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509
Signature:
Date 10/5/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Prenatal developmental study - Rabbit; OPPTS 870.3700; OECD 414.

PC CODE: 005100 005209 **DP BARCODE**: D305670

TEST MATERIAL (PURITY): GF-871 (41.3% XDE-750 TIPA salt in water)

SYNONYMS:

XDE-750 triisopropanolammonium, XDE-750 TIPA, aminopyralid triisopropanolammonium, X677349

CITATION: Carney, EW, and B Tornesi, March 05, 2004. GF-871: Oral gavage developmental toxicity study in New Zealand White rabbits. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 031142; Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN

EXECUTIVE SUMMARY:

In a teratogenicity study in New Zealand White rabbits (MRID 46235632), GF-871 (41.3 % XDE-750 triisopropanolammonium, TIPA) in deionized water was administered by oral gavage to groups of 26 mated females at dose levels of 0, 484, 1211, or 2421 mg/kg bw/d (0, 200, 500, or 1000 mg/kg bw/d based on XDE-750 TIPA) at a constant volume of 4 mL/kg bw on gestation days (GD) 7-27. The acid (ae) equivalent doses are 0, 104, 260, 520 mg ae/kg bw/d. The day when copulation was observed was designated as day 0 of gestation. In-life maternal parameters examined included clinical observations, body weight, body weight gain, and feed consumption. On GD 28, all survivors were euthanized and examined for gross pathologic alterations and changes in liver, kidney, and gravid uterine weights. The numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses were determined. All fetuses were weighed, sexed and examined for external, visceral, and skeletal abnormalities. Also, the internal structures of the head were examined by serial sectioning for approximately one-half of the fetuses in each litter. At 1000 mg/kg bw/d, maternal toxicity was evidenced by decreased feed consumption and body weight gains. One high-dose rabbit aborted (one control also aborted), and three others were euthanized early due to severe inanition and subsequent body weight loss. At 500 mg/kg bw/d, one rabbit was euthanized due to severe inanition and body weight loss. Treatment-related clinical observations included decreased fecal output in the 500 and 1000 mg/kg bw/d groups, and signs of mild incoordination in 0, 1, 2, and 19 rabbits from the 0, 200, 500, and 1000 mg/kg bw/d dose groups, respectively. The incoordination was transient, occurred sporadically, and in the 500 and 200 mg/kg bw/d groups, was seen only once in each affected rabbit. Developmental effects were limited to decreased fetal body weights at 1000 mg/kg bw/d.
There were no evidence of teratogenicity. Based on these results, the maternal LOAEL is 500 mg/kg bw/day (260 mg ae/kg bw/d). The NOAEL for maternal toxicity is 200 mg/kg bw/d (104 mg ae/kg bw/d). The developmental LOAEL is 1000 mg/kg bw/d (520 mg ae/kg bw/d) and the developmental NOAEL is 260 mg ae/kg bw/d.

The developmental toxicity study in the rabbit is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (DACO 4.5.3; OPPTS 870.3700; OECD 414) in rabbits.

COMPLIANCE:

Signed GLP, Quality Assurance, and Data Confidentiality statements were submitted.

This study was conducted according to the following test guidelines: USEPA: United States Environmental Protection Agency, Health Effects Test Guidelines. OPPTS 870.3700, Prenatal Developmental Toxicity Study August, 1998; OECD: Organisation for Economic Co-Operation and Development. Guidelines for Testing of Chemicals, Section 4: Health Effects, Protocol Number 414, Prenatal Developmental Toxicity, 2001.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test material:** GF-871

Description:	wine coloured liquid
Lot/Batch #:	173-162-IA/TSN10410
Purity:	41.3% XDE-750 TIPA salt in water
Compound stability:	aqueous solutions of GF-871 at 50 and 250 mg/mL were stable for ≥22 days
CAS #:	150114-71-9 and 000122-20-3

2. **Vehicle control:** Deionized water

3 **Test animals:**

Species:	rabbit, time-mated females	
Strain:	New Zealand White	
Age/weight at study initiation:	5-6 months, 2500-3500 g	
Source:	Covance Research Products, Inc. (CRP), Kalamazoo, MI	
Housing:	singly in suspended stainless cages with flattened tube grid floors	
Diet:	LabDiet. Certified Rabbit Diet #5325 (PMI Nutrition International, St. Louis, MO) in pelleted form *ad libitum*	
Water:	municipal tap water *ad libitum*	
Environmental conditions:	**Temperature:**	22±2 °C
	Humidity:	40-60 %
	Air changes:	12-15 changes/h
	Photoperiod:	12 h dark / 12 h light
Acclimation period:	~6 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - Start: September 22, 2003 End: November 5, 2003

2. **Mating:**

Sexually mature virgin females were naturally mated with males of the same strain. The observed day of breeding was considered day 0 of gestation. Rabbits were shipped on days 0 or 1 of gestation.

3. **Animal assignment:**

Animals were assigned by randomly using a computer program to test groups as indicated in Table 1

Table 1: Animal assignment

mg GF-871/kg bw/d	0	484	1211	2421
mg XDE-750 TIPA/kg bw/d	0	100	500	1000
mated	26	26	26	26

4. Dose selection rationale:

The dose levels were chosen in an attempt to produce graded responses to the test article.

5. Dosage preparation and analysis:

GF-871 was administered in deionized water at a dose volume of 4 mL/kg bw to yield the targeted dose. Dose volumes were adjusted daily based on individual body weights. Due to the length of the dosing period, dose solutions were prepared periodically throughout the study.

Analysis of all dosing suspensions from the first mix were initiated prior to the start of dosing using HPLC with ultraviolet detection and external standards to determine concentrations.

Results:

Stability analysis:
Aqueous solutions of GF-871 at concentrations of 50 and 250 mg/mL were stable for at least 22 days. The concentrations after storage were 96.0 to 102 % of the initial concentrations.

Concentration analysis:
Analyses of all dosing solutions revealed concentrations of 101 to 107 % of the target levels.

Stability and concentration analysis data indicated that the mixing procedures and dosing preparations were adequate and acceptable.

6. Dosage administration:

Animals were dosed by oral gavage from day 7 to 27 of gestation at a constant volume of 4 mL/kg bw. Dosing was based on the body weight on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal observations and evaluations -

The animals were checked daily for mortality or clinical signs. Body weight was recorded on gestation day 0, and daily from day 7-28. Food consumption data were recorded on GD 4-28. Moribund animals that were not expected to survive until the next observation period, and any animals found dead, were necropsied on that day. However, animals found dead after routine working hours or on weekends and holidays were refrigerated until the next scheduled workday, at which time they were necropsied. For rabbits with premature delivery, the delivered fetuses were counted and examined to the extent possible. Dosing was discontinued, and animals were euthanized and necropsied on that day or, if found after regular working hours, the next scheduled workday. On GD 28, all pregnant rabbits were killed by iv injection of Beuthanasia-D and necropsied. The weights of the liver, kidneys, and gravid uterus were determined. The uterine contents were examined for corpora lutea, implantations, and live and dead fetuses.

2. Fetal evaluations

All fetuses were weighed and, after euthanized by sublingual oral administration of sodium pentobarbital solution, examined for external, visceral, and skeletal abnormalities. The fetuses were sexed by examination of the gonads. Approximately one half of the fetuses in each litter were randomly selected for craniofacial examination. The heads of these fetuses were removed, placed in Bouin's fixative and serially sectioned to allow for inspection of the eyes, brain, nasal passages, and tongue. All fetuses were then eviscerated, preserved in alcohol and stained with Alizarin Red S in order to visualize ossified bone. After staining, skeletons were macerated and cleared, and a thorough evaluation of the fetal skeleton was conducted. All fetal alterations were classified as a variation or malformation. A variation was defined as a divergence beyond the normal range of structural constitution that may not adversely affect survival or health. A malformation was defined as a permanent structural change that may have adversely affected survival, development or function and/or which occurred at a relatively low incidence in the specific species/strain.

D. DATA ANALYSIS

1. Statistical analyses:

Maternal body weights, body weight gains, organ weights (absolute and relative), fetal body weights and feed consumption were evaluated by Bartlett's test ($\alpha = 0.01$) for equality of variances. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant at $\alpha = 0.05$, analysis by Dunnett's test ($\alpha = 0.05$) or the Wilcoxon Rank-Sum test ($\alpha = 0.05$) with Bonferroni's correction was performed, respectively. To investigate the possible causes of an apparent effect on fetal body weights, a post-hoc analysis of covariance (litter size x pup body weight) was also conducted. Pre- and post-implantation loss, and fetal alterations were analyzed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea, implantations, and litter size were evaluated using a non-parametric ANOVA ($\alpha = 0.05$) followed by the Wilcoxon Rank-Sum test ($\alpha = 0.05$) with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test ($\alpha = 0.05$) with Bonferroni's correction. Fetal sex ratios were analyzed using a binomial distribution test. Females lacking visible implantations at the scheduled necropsy were excluded from the appropriate analyses. Statistical outliers were identified, using a sequential method ($\alpha = 0.02$). Both Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control group to keep the experiment-wise α at 0.05. Both were reported at the experiment-wise α level.

2. Indices:

The following indices were calculated from cesarean section records of animals in the study:

- Pre-implantation loss* = [(No. corpora lutea-implantations) x 100] / No. corpora lutea
- Post-implantation loss* = [(No. implantations – viable fetuses) x 100] / No. implantations

* Note: Percent pre- and post-implantation loss was determined for each litter, followed by calculation of the mean of these litter values.

3. Historical control data: Historical control data were provided for fetal body weights..

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical observations: Table 2.

Examinations performed on all rabbits prior to the start of dosing (GD 0-6) revealed incidences of abrasions, scratches and/or swellings (located on the ear flaps, hock, back, or vulva), which were

likely inflicted during breeding at the supplier's facility.

During the dosing period, absent and/or decreased amounts of feces associated with severe inanition and body weight loss were noted in a number of rabbits. Due to the magnitude and duration of these effects, one mid-dose and three high-dose rabbits were euthanized between GD 14-18. At necropsy, all four of these rabbits were found to be pregnant. One high-dose rabbit exhibited lung edema, congestion of the liver and lung, and a hairball in the stomach; the other two high-dose animals had no visible lesions. The mid-dose rabbit had congestion of the liver and a hairball in the stomach. One control and one high-dose animal aborted (blood and fetal tissue were found their cages) on GD 27 and 26, respectively. They were euthanized and examined grossly at necropsy. Gross necropsy showed pale liver, perineal soiling, and a stomach ulcer in the control rabbit; and in the high-dose rabbit, watery contents of the gastrointestinal tract, pale liver, and very dark urine in the urinary bladder. The causes of the abortions were not determined.

One control and one high-dose animals died spontaneously; both of them were pregnant. The control rabbit died on GD 17. Gross findings in this animal revealed laceration of the esophagus accompanied by muscle inflamation and edema in the neck area, gas in the gastrointestinal tract, and facial and perineal soiling. The high-dose rabbit died on GD 7, necropsy of which revealed generalized edema of the lungs and froth in the trachea. The cause of death for both animals was attributed to gavage complication.

In addition to the clinical observations mentioned above, signs of incoordination were noted in 19 of 26 high-dose rabbits. This incoordination presented itself as slight stumbling behavior when the rabbits were trying to walk or turn in their cages, and appeared to affect the forelimbs only. The rabbits appeared normal when sitting upright or lying recumbent in their cages. Incoordination was transient, occurred sporadically, and highly variable for its first appearance. Clinical signs in rabbits can be difficult to interpret. These signs of incoordination were very similar to those observed in previous rabbit studies with XDE-750 acid. Six high-dose rabbits exhibited repetitive chewing behavior that was transient, and in most of the cases, noted on 1-2 consecutive days only. Two mid-dose rabbits and one low-dose animal displayed single incidences of transient incoordination during the course of the study. Decreased activity was also noted in one of these mid-dose rabbits on the same day the incoordination was observed. Two high-dose animals also were cold to the touch, but these rabbits did not exhibit signs of incoordination. Other clinical signs noted in high- and mid-dose animals included absent and/or decreased amounts of feces, which were temporally correlated with a reduction in feed consumption. In the low-dose group, there as a slight increase in the number of rabbits with a reduced feces; but this was seen for only a very brief period (approximately GD 15-16), being temporally associated with slight decreases in feed consumption during the same period. Thus, this finding in the low-dose group rabbits was not considered toxicologically significant.

Table 2: Clinical signs

mg GF-871/kg bw/d	0	484	1211	2421
mg XDE-750 TIPA/kg bw/d	0	100	500	1000
mated	26	26	26	26
within normal limits	15	13	9	0
unscheduled sacrifice	0	0	1	3
faeces - absent - decreased - soft - watery	1300	610	21042	**51820**
gait - incoordination	0	1	2	**19**

repetitive chewing behaviour	0	0	0	6
cold to touch	0	0	0	2
Data taken from Table 2, pp 30-32 of Report				

2. **Body weight** - Body-weight data are presented in Table 3.

Body weight gains of high-dose rabbits were significantly lower relative to controls on GD 7-10, 10-13, 13-16, and 7-28 (Table 3). Body-weight gains of mid-dose rabbits were generally lower than the control rabbits from GD 7 to 16. Lower body-weight gains of these animals were generally associated with lower food intake during the same periods. Body-weight gains of low-dose rabbits were comparable to control rabbits.

Table 3 : Maternal body-weight gains (g±SD, /N)

mg/kg bw/d	0	200	500	1000
mated ♀	26	26	26	26
days 0-7	116.5±128.8	130.5±107.3/25	102.7±107.0/24	118.8±155.3/23
38542	20.1±40.2	12.7±44.5	-8.2±58.5	**-69.5±87.3/22**
38637	59.5±36.2	41.3±38.5	23.7±59.9	**-15.8±71.9**
38732	80.8±40.7	72.6±59.3	52.1±61.4	**26.8±70.3/20***
16-20	51.8±38.2/25	45.6±59.0	48.4±50.9	51.2±78.7/19
20-24	63.0±31.2	67.6±35.4	68.8±60.3	74.3±49.0
24-28	33.5±69.9/24	36.4±52.3/24	55.2±30.6	45.4±46.7/17
38560	311.1±89.1	278.4±111.2	255.7±101.1	**180.3±152.9**
gain days 0-28	425.0±165.1	411.4±186.3	360.5±179.9/23	**309.4±146.4**
shaded period = treatment period; * p<0.05; data taken from Table 4, page 34 of Report				

3. **Food consumption** - Food consumption data are presented in Table 3.

Feed consumption in treated groups was comparatively lower when compared to controls and was dose-related. Specifically, decreases in feed consumption were statistically identified in the high-dose group from GD 7-21, in the mid-dose group from GD 13-17 and 18-19, and in the low-dose group from GD 13-15. Whereas the decreased feed consumption in the high-dose animals was accompanied by decreases in body weight gains, the decreased feed consumption in the mid- and low-dose groups had no appreciable impact on body weights or body weight gains.

Table 3 : Food consumption data (g/rabbit/d±SD, /N)

mg/kg bw/d	0	200	500	1000
mated ♀	26	26	26	26
days 6-7	188.8±38.9	186.2±37.2/24	185.1±40.4/25	197.9±22.4/23
38540	184.1±34.7	179.2±35.6/25	164.5±42.5/25	**116.9±63.9/22***
38604	180.4±31.2	170.7±38.3	156.3±53.9	**121.2±60.2***

38635	174.2±37.4	163.7±37.2	149.0±56.0	**113.8±63.3***
38698	155.3±45.5	140.3±48.7	121.6±55.0	**69.2±64.7***
14-15	170.6±30.9	**127.6±64.1***	**109.3±67.1***	**72.7±68.2***
16-17	187.2±35.2/25	166.0±39.2	146.4±63.2	**111.2±67.6***
18-19	190.9±37.2	175.5±33.6	**157.2±47.3/24***	**126.8±58.9/20***
20-21	189.2±40.7	171.0±25.8	166.4±39.0	**150.2±45.1/19***
22-23	169.0±37.7	155.4±31.7	147.4±37.5	143.6±45.2
24-25	148.5±37.3	143.8±33.9	140.6±25.1	131.9±42.8
26-27	144.4±43.3/24	138.6±34.7	138.1±17.7	137.6±26.4/18
27-28	146.1±43.2	144.6±32.5	143.5±24.9	132.2±41.8
shaded period = treatment period; * p<0.05; data taken from Table 5, pp 35-36 of Report				

4. **Gross pathology** - There were no treatment-related findings.

5. **Organ weights** -

There were no treatment-related effects on the weights of the liver, kidneys, or gravid uterus.

6. **Cesarean section data** - Data are presented in Table 5.

There were no treatment-related findings on the numbers of corpora lutea, pre- and post-implantation loss, sex ratio. Fetuses from the high-dose group had lower body weights relative to controls, statistically significant for female fetuses and for both sexes combined. In light of the fact that mean litter sizes were slightly larger in the high-dose group (8.6±2.1 fetuses) relative to the control group (7.5±2.6 fetuses), and that fetal body weights vary inversely with litter size, a post-hoc analysis of covariance was conducted to determine if the fetal body weight decreases in the high-dose group were due to the larger litter sizes in this group. However, the effect of GF-871 on fetal body weight remained significant even after considering the influence of litter size. Furthermore, mean fetal body weights of the high-dose males, females and both sexes combined were outside of recent historical control ranges. Therefore, the effects on fetal body weights at the high-dose were considered treatment-related. No effects on fetal body weights or any other reproductive parameters were observed in the mid- and low-dose groups.

Table 5 : Cesarean section observations

mg/kg bw/d	0	200	500	1000	historical
mated ♀	26	26	26	15	
♀ died	1	0	0	0	
pregnant ♀	26	25	25	23	
fertility index (%)	26/26(100)	25/26 (96.2)	25/26 (96.2)	23/26 (88.5)	
aborted	1	0	0	1	
terminated early (inanition)	0	0	1	3	
♀ with viable litter, N	24	25	24	18	

corpora lutea / dam	9.4±2.0	9.6±1.6	9.7±1.8	10.3±2.4	
implantations / dam	7.6±2.7	8.6±2.2	8.4±1.8	9.2±2.0	
pre-implantation loss/dam	1.8±2.2	1.0±2.1	1.3±1.3	1.1±1.5	
post-implantation loss, #/%	1.9±4.4	4.0±6.8	2.9±6.0	6.8±10.1	
live foetuses / dam	7.5±2.6	8.2±2.2	8.2±1.9	8.6±2.1	
sex ratio, ♂/♀	49 / 51	51 / 49	45 / 55	54 / 46	
gravid uterus weight, g	420.0±122.0	457.7±103.4	451.0±88.2	459.6±79.0	
fetal body weight, g, ♂	38.3±4.5	37.7±3.6	37.4±3.6	35.4±3.6	35.89-38.31
♀	37.6±4.0	36.0±3.0	36.6±3.8	**33.6±3.6***	35.73-36.52
♂♀	38.3±4.0	37.0±3.5	37.0±3.4	**34.5±3.6***	35.71-37.34
* $p<0.05$; data taken from Table 8, page 45 of Report; historical control data were based on 3 studies conducted during 2000-2002					

B. DEVELOPMENTAL TOXICITY

There were no treatment-related external, visceral, and skeletal abnormalities. Malformed fetuses are presented in Table 6. The observed malformations lacked a dose-response relationship and consistent patterns, the findings were considered spurious and unrelated to treatment with GF-871.

Table 6 : Fetal malformation data

mg/kg bw/d	0	200	500	1000
fetuses/litters examined	179 / 24	207 / 25	196 / 24	155 / 18
external abnormalities	0 / 0	0	0 / 0	0
visceral malformations:				
missing brain ventricle	1/ 1	0 / 0	0 / 0	0 / 0
missing pulmonary valve	0 / 0	1 / 1	0 / 0	0 / 0
misshapen heart, general	0 / 0	1 / 1	0 / 0	0 / 0
misshapen semilunar valve	0 / 0	1 / 1	0 / 0	0 / 0
misshapen ventricle	0 / 0	0 / 0	0 / 0	1 / 1
persistent truncus arteriosus aortic arch	0 / 0	2 / 2	0 / 0	0 / 0
stenosis of pulmonary artery	0 / 0	0 / 0	0 / 0	1 / 1
missing gall bladder	0 / 0	0 / 0	1 / 1	0 / 0
ectopic kidney	1 / 1	0 / 0	0 / 0	0 / 0
misshapen kidney	1 / 1	0 / 0	0 / 0	0 / 0
skeletal malformations:				
fused frontal	1 / 1	0 / 0	0 / 0	0 / 0
extra thoracic vertebrae	0 / 0	1 / 1	0 / 0	0 / 0
hemi-vertebra lumbar vertebrae	0 / 0	1 / 1	0 / 0	0 / 0
missing lumbar vertebrae	0 / 0	0 / 0	1 / 1	0 / 0
extra thoracic centra	0 / 0	1 / 1	0 / 0	0 / 0
missing lumbar centra	0 / 0	0 / 0	1 / 1	0 / 0
extra thoracic rib	0 / 0	1 / 1	1 / 1	0 / 0
forked ribs	0 / 0	1 / 1	0 / 0	0 / 0
fused thoracic rib	0 / 0	0 / 0	1 / 1	0 / 0
total malformed fetuses / litters	1 / 1	3 / 3	2 / 2	2 / 2
data taken from Table 9, pp 46-53 of Report				

III. DISCUSSION

A. Investigators' conclusions:

"In conclusion, 1000 mg/kg/day XDE-750 TIPA caused maternal toxicity as evidenced by decreased feed consumption and body weight gains. One high-dose rabbit aborted (one control also aborted), and three others were euthanized early due to severe inanition and subsequent body weight loss. At 500 mg/kg/day, one rabbit was euthanized due to severe inanition and body weight loss. Treatment-related clinical observations included decreased fecal output in the 500 and 1000 mg/kg/day groups, and signs of mild incoordination in 0, 1, 2, and 19 rabbits from the 0, 200, 500, and 1000 mg/kg/day dose groups, respectively. The incoordination was transient (resolving within 1-2 hours postdosing), occured sporadically, and in the 500 and 200 mg/kg/day groups, was seen only once in each affected rabbit. The low incidence at 200 mg/kg/day (1 of 26) suggests that this dose level is at or near the limit of detection for this maternal effect. Developmental effects were limited to decreased fetal body weights at 1000 mg/kg/day. There were no other effects on fetal development at any dose level. Based upon these results, the no-observed-adverse effect-level (NOAEL) for maternal toxicity is expected to be just slightly below 200 mg/kg/day. The NOEL for developmental toxicity was 500 mg/kg/day."

B. Reviewer's discussion: The study was properly conducted and the authors' conclusions are valid.

C. Study deficiencies: There were no deficiencies.

APPENDIX: Prenatal Developmental Toxicity Study-supplemental report for GF-871

TEST MATERIAL PURITY: GF-871 (41.3% XDE-750 TIPA salt in water)

CITATION: Carney, EW, and B Tornesi, May 24, 2004. GF-871: Oral gavage developmental toxicity study in New Zealand White rabbits. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 031142S; Unpublished.

In a supplemental teratogenicity study in New Zealand White rabbits (MRID 46284901), GF-871 (41.3 % XDE-750 triisopropanolammonium, TIPA) in deionized water was administered by oral gavage to groups of 26 mated females at dose levels of 0, 50, or 150 mg/kg bw/d (expressed in terms of XDE-750 TIPA)on gestation days (GD) 7-27. The acid (ae) equivalent doses are 0, 26, and 78 mg ae/kg bw/d. In-life maternal parameters examined included clinical observations (performed blind to treatment group identity), body weight, body weight gain, and feed consumption. On GD 28, all survivors were euthanized and examined for gross pathologic alterations and pregnancy status.

Over the course of the 21-day dosing period, transient (lasting 30-75 minutes post-dosing) incoordination was seen only once in each of three rabbits in the 150 mg/kg bw/d group. In two of the affected animals a repetitive chewing behavior was also observed concomitant with the incoordination. There were no treatment-related clinical signs observed in the 50 mg/ kg bw/d group. There were no treatment-related effects on body weight, body weight gain, feed consumption, or gross necropsy at any dose level.

The numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses were determined. All fetuses were weighed, sexed and examined for external, visceral, and skeletal abnormalities. Also, the internal structures of the head were examined by serial sectioning for approximately one-half of the fetuses in each litter. At 1000 mg/kg bw/d, maternal toxicity was evidenced by decreased feed consumption and body weight gains. One high-dose rabbit aborted (one control also aborted), and three others were euthanized early due to severe inanition and subsequent body weight loss. Based on the results, **the NOAEL for maternal effects is 50 mg/kg bw/d (26 mg ae/kg bw/d) and the LOAEL is 150 mg/kg bw/d (78 mg ae/kg bw/d).**

005100 (7)

PMRA Sub. No. 2004-0789/DOW AMINOPYRALID/AMD — ~ PROTECTED ~ — Reproduction Study / 1
DACO 4.5.1 / OECD IIA 5.6.1

PMRA Primary Reviewer: Brenda MacDonald, DVM
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: for
Date Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509
Signature:
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - rat, OPPTS 870.3800; OECD 416.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Marty, M.S., et al (2003) XDE-750: Two-Generation Dietary Reproduction Toxicity Study in CD Rats. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011205, June 16, 2003. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a 2-generation rat reproduction study (MRID 46235635), XDE-750, purity 94.5%, was administered to 30 Sprague Dawley rats per sex per group in the diet at concentrations of 0, 50, 250 or 1000 mg/kg bw/day (equal to 0, 52.0, 259 or 1030 mg/kg bw/day for males, and 0, 49.3, 245 or 973 mg/kg bw/day for females). Each female in each generation was mated to produce one litter. For parent animals, there were no treatment-related effects on mortality, clinical signs, body weight and body weight gain, food intake, reproductive function, reproductive parameters or histopathology. Full and/or empty cecal weights were increased in the P_1 generation, in the 250 and 1000 mg/kg bw/day groups, both sexes. In the P_2 generation, full and empty cecal weights were increased in the 1000 mg/kg bw/day group, both sexes, and in the 50 and 250 mg/kg bw/day groups, males only. At gross necropsy, cecal size was increased in the P_1 and P_2 generations, in the 250 and 1000 mg/kg bw/day groups, both sexes. In the absence of any histopathological changes to the ceca, and in the absence of any other treatment-related parental findings, the cecal findings were considered to be adaptive changes and were not considered to be adverse.
For pups, there were no treatment-related effects on clinical signs, viability/litter parameters, pup body weight and body weight gain, organ weights or gross pathology

For parental toxicity, the LOAEL could not be determined since there were no adverse, treatment-related effects. The NOAEL is 1000 mg/kg bw/day (1030/973 mg/kg bw/day).

For reproductive toxicity, the LOAEL could not be determined since there were no treatment-related effects. The NOAEL is 1000 mg/kg bw/day (1030/973 mg/kg bw/day).

For offspring toxicity, the LOAEL could not be determined since there were no treatment-related effects. The NOAEL is 1000 mg/kg bw/day (1030/973 mg/kg bw/day).

The 2-generation reproduction study in the rat is classified acceptable and satisfies the guideline requirement for a reproduction toxicity study (OPPTS 870.3800) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description:	Technical; tan powder.
Lot/Batch #:	F-0031-143; TSN102319
Purity:	94.5% a.i.
Compound Stability:	Not stated.
CAS #:	150114-71-9

2. **Vehicle:** Test material was mixed with control diet (Purina #5002 Certified Rodent Diet).

3 **Test Animals:**

Species:	Rat	
Strain:	CD (Crl:CD (SD) IGS BR	
Age at study initiation:	Approximately 6 weeks of age.	
Wt. at study initiation:	Males: 174.5 g to 214.1 g; Females: 133.2 g to 164.9 g.	
Source:	Charles River Laboratories, Inc., Portage, Michigan.	
Housing:	Individually housed in stainless steel cages. Dams were housed individually in plastic cages provided with corn cob nesting material from day 19 of gestation throughout lactation.	
Diet:	LabDiet Certified Rodent Diet #5002 in meal form, *ad libitum*	
Water:	Municipal water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	40-70%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 2 weeks.	

B. PROCEDURES AND STUDY DESIGN

1. **Mating Procedure:** Male and female rats were fed test diets for 10 weeks prior to mating. Females were mated with males from the same test group, 1:1 ratio, until successful mating occurred, or for 2 weeks (whichever came first). Mating was confirmed by the presence of a copulatory plug in the vagina or sperm in the vaginal smears, and was designated as day 0 of gestation. Both the P_1 and P_2 generations were mated to produce 1 litter each, i.e., F_1 and F_2, respectively.

2. **Study Schedule:** Starting at 8 weeks of age, P_1 generation animals were given test diets for 10 weeks before they were mated. F_1 weaned offspring (day 21 post-partum) were selected randomly to become P_2 generation parents (one rat/sex/litter, if available). Cohabitation of F_1 male and female littermates was avoided.

3. **Animal Assignment:** Animals were stratified by body weight and then randomly assigned to the test groups noted in Table 2 using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of the study.

TABLE 1 - Animal Assignment

Test Group	Dose in Diet[a]	Animals/group			
		P_1 Males	P_1 Females	P_2 Males	P_2 Females
Control	0	30	30	30	30
Low (LDT)	50	30	30	30	30
Mid (MDT)	250	30	30	30	30
High (HDT)	1000	30	30	30	30

[a] Diets were administered from the beginning of the study until sacrifice.

4. **Dose Selection Rationale:** Dose levels were selected based on the previous 13-week dietary probe study in CD rats. Administration of XDE-750 at the high dose (1000 mg/kg bw/day) was expected to produce increases in cecum weights with possible epithelial hyperplasia of the cecum (based on the reproductive toxicity probe study described below). The top dose level of 1000 mg/kg/day for this study was a limit dose as defined by the relevant regulatory guidelines. The middle- and low-doses were expected to provide dose response data for any treatment-related effects observed in the high-dose group and to establish a no observed-effect level (NOEL). In the probe study, groups of 10 male and 10 female CD (Sprague-Dawley strain) rats were fed diets providing 0, 100, 500, or 1000 mg XDE-750/kg body weight/day for 13 weeks to evaluate the potential toxicity of XDE-750, an experimental herbicide, in this strain of rats (Liberacki *et. al.* 2001). Males and females administered 500 or 1000 mg XDE-750/kg bw/day had treatment-related effects on the cecum and ileum. The absolute and relative weights of ceca (full and empty) were significantly increased in males and females given 500 or 1000 mg/kg bw/day. Treatment-related gross pathologic findings in males and females given 500 and 1000 mg/kg bw/day were limited to increased size of the cecum. Histopathologic alterations in animals given 1000 mg/kg bw/day were confined to the cecum (males and females) and ileum (males only), which were characterized by very slight diffuse hyperplasia of the crypt epithelium of the respective mucosa. The cecal hyperplasia correlated with statistically identified increases in mean cecum weights (full and empty) of males and females given 1000 mg/kg bw/day. There was no histopathologic correlate to the statistically identified increase in cecum weights of males and females given 500 mg/kg bw/day. No treatment-related effects were noted in either sex administered 100 mg/kg bw/day. Therefore, under the conditions of this study, the no-observed-effect level (NOEL) for male and female CD rats was 100 mg/kg bw/day.

5. **Dosage Preparation and Analysis:** Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed (LabDiet Certified Rodent Diet #5002). Pre-mixes were prepared periodically throughout the study based on stability data. Diets were prepared weekly for approximately 10 weeks prior to the breeding of the P_1 animals. The concentration of the test material in the diets were calculated from the most recent body weight and feed consumption data. Initial concentrations of test material in the diet were calculated from pre-exposure body weights and feed consumption data. To avoid potential overdosing during the breeding period, animals co-housed were provided with the lower of the two concentrations (female diet) for that dose group (low, middle, or high). During gestation, females from each dose group were provided with the appropriate dietary concentration of XDE-750 given during breeding. Dietary concentrations supplied during lactation were adjusted using historical control feed consumption data for lactating females to account for the large and rapid increase in feed consumption (2-3x increase) typical for rats in late lactation. Until all litters were weaned, weanlings received a diet containing the same concentration of XDE-750 that was given to the P_1 females during the third week of lactation. Dams awaiting necropsy received a diet containing the

same concentration of XDE-750 that was given during the breeding period until all litters had finished the lactation phase. Dietary concentrations for the P_2 generation were calculated as described for the P_1 animals. Stability of the test material in diet for up to 21 days was demonstrated in the 4-week dietary toxicity study in mice (Study No. 001048). In addition, stability up to 55 days and 35 days was shown in the 2-year rat chronic toxicity/oncogenicity/chronic neurotoxicity study and 18-month mouse chronic toxicity/oncogenicity study, respectively. Hence, stability analyses were not conducted in the reproduction study. Homogeneity of mixing was determined for the low-dose and high-dose diets at the start of the study, approximately mid-way through the study and near study termination. Actual test material concentration in the diet was determined for all dose levels from test diets prepared at the start of the study, approximately mid-way through the study and near the end of the study.

Results - Homogeneity Analysis: Analyses confirmed that the test material was homogeneously distributed in the diets. Percent relative standard deviation (RSD) values, which were calculated from the variance between top and bottom concentrations within test diets, ranged from 1.79 to 7.41%.

Stability Analysis: The stability of XDE-750 in rodent chow was determined in previous toxicity studies. A previous four-week toxicity study in mice demonstrated XDE-750 to be stable for at least 21 days in rodent chow at concentrations ranging from 0.005% to 3%. An on-going two-year dietary chronic toxicity/oncogenicity and chronic neurotoxicity study has demonstrated XDE-750 to be stable for at least 55 days at a 7% concentration. An 18 month chronic toxicity/oncogenicity study has demonstrated XDE-750 to be stable for at least 35 days at 0.0258%. Test diets for the current study were prepared and used within these stability limits.

Concentration Analysis: Individual samples of the 50, 250 and 1000 mg/kg bw/day test diets ranged from 83.3% to 117%, 95.7% to 117% and 97.4% to 106% of the nominal concentrations, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. Parental Animals: Clinical examinations were conducted on all males pre-study and weekly thereafter. Clinical examinations were conducted on all females pre-exposure and weekly throughout the pre-breeding and breeding periods. Mated (sperm- or plug-positive) females received clinical examinations on gestation days 0, 7, 14 and 21. In addition, mated females were observed for signs of parturition beginning on or about day 20 of gestation. Females that delivered litters were subsequently evaluated on lactation days 0, 1, 4, 7, 14 and 21. Females that failed to mate or failed to deliver litters were examined weekly. Examinations included a careful, hand-held evaluation of the skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), swellings, masses, and animal behavior. Twice each day a cage-side examination was conducted with the exception of one day (4/18/02) when an afternoon cageside examination was inadvertently not conducted. To the extent possible the following parameters were evaluated: skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), animal behavior, moribundity, mortality, and the availability of feed and water. Moribund animals not expected to survive until the next observation period, and any animals found dead were necropsied on that day. Animals found dead after routine working hours or on weekends or holidays were refrigerated until the next scheduled work day at which time they were necropsied. For males, individual body weights were measured on a weekly basis throughout the study period, and food consumption was measured on a weekly basis during the pre-mating and post-mating periods. For females, individual body weights were measured weekly during

premating, on days 0, 7, 14 and 21 of gestation and on days 1,4, 7, 14 and 21 of lactation; food intake was measured weekly during premating and on days 0 7, 14 and 21 of gestation, and on days 1, 4, 7, 11, 14, 17, 19 and 21 of lactation.

During the last 3 weeks of the pre-mating period, during cohabitation until each female was sperm-positive and on the day of scheduled necropsy, slides of the estrous cycles were prepared from vaginal lavage samples, which were examined to determine estrous cycle length and pattern.

2. Litter Observations: On day 4 postpartum, all litters were culled by random selection to a maximum of 8 pups/litter (4/sex/litter, if possible); excess pups were killed and discarded. Litter parameters measured and recorded were duration of gestation; external abnormalities at birth; the number of live and dead pups on days 0, 1, 4, 7, 14 and 21, and the sex and weight of each pup on days 0, 1, 4 (pre- and post-cull), 7, 14 and 21 post-partum. All F_1 weanlings selected for mating were observed daily for vaginal opening beginning on postnatal day 28 or preputial separation beginning on day 35. The age and body weights at the time of landmark acquisition were recorded. Because there was not a treatment-related effect on the F1 sex ratio, age at vaginal opening or age at preputial separation, anogenital distance was not measured in the F_2 pups. Dead/moribund pups were sexed, examined grossly for external and internal abnormalities, and then preserved in neutral, phosphate-buffered 10% formalin.

3. Postmortem Observations:

1) Parental Animals: All males and pregnant females which gave birth were sacrificed as close as possible to the weaning of the last litter by decapitation while under CO_2 anesthesia. All animals were necropsied, including those found dead. The uteri of all females were stained with an aqueous solution of 10% sodium sulfide stain for approximately 2 minutes and were examined for the presence and number of implantation sites. Uteri from females that did not deliver a litter and had no visible implantation sites were examined for evidence of early resorption in order to verify pregnancy status. After evaluation, uteri were gently rinsed with saline and preserved in neutral phosphate-buffered 10% formalin.

The following tissues (✓) were prepared for microscopic examination:

✓	Ovaries	✓	pituitary
✓	Uterus, with oviducts and cervix	✓	liver
✓	testes	✓	kidneys
✓	epididymides	✓	adrenal glands
✓	seminal vesicles with coagulating glands	✓	spleen
✓	prostate	✓	cecum (empty and full)
✓	brain	✓	thyroid

These tissues were examined histopathologically for all animals in the control and high dose groups, including relevant gross lesions. Reproductive organs of low- and mid-dose animals with signs of reduced fertility were also examined histopathologically. Sperm analysis was conducted, including motility, sperm counts, morphology and histopathology. A qualitative evaluation of the testes included examination for retained spermatids, missing germ cell layers or types, multinucleated giant cells and sloughing of spermatogenic cells into the lumen.

2) Offspring: The F_1 offspring not selected as parental animals and all F_2 offspring were sacrificed at 22 days of age. These animals (3 pups/sex/litter when permitted by litter size) were subjected to postmortem macroscopic examination as described above for adults. One pup/sex/litter was randomly selected for the collection of brain, spleen, uterus and thymus weights. Organ-to-body weight ratios were calculated. Representative samples of grossly abnormal tissues were collected from all weanlings at the scheduled

necropsy. In addition, the brain, spleen, thymus, cecum and ileum were saved for the weanlings selected for organ weight measurements in the event that an effect on organ weight was observed and/or future evaluation was desirable. Tissues were fixed in neutral phosphate-buffered 10% formalin. There were no treatment-related gross lesions in the F_1 or F_2 weanlings and no effects on weanling organ weights; therefore, histological examination was not conducted.

D. DATA ANALYSIS

1. Statistical Analyses: Parental body weights, gestation and lactation body weight gains (females only), litter mean body weights, feed consumption, sperm count, follicle count, percent total and progressively motile sperm, mean estrous cycle length, and organ weights (absolute and relative) were first evaluated by Bartlett's test (alpha = 0.01) for equality of variances. Based upon the outcome of Bartlett's test, either a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha = 0.05, a Dunnett's test (alpha = 0.05) or the Wilcoxon Rank-Sum (alpha = 0.05) test with Bonferroni's correction was performed.
Gestation length, age at vaginal opening (females), age at preputial separation (males), average time to mating, and litter size were analyzed using a nonparametric ANOVA. If the ANOVA was significant, the Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Sperm morphology data were arcsine transformed and analyzed by a parametric ANOVA. If the ANOVA was significant, the Dunnett's test was performed. Statistical outliers (alpha = 0.02) were identified by the sequential method of Grubbs and were routinely excluded from feed consumption only. Other outliers were only excluded from analysis for documented, scientifically sound reasons. The mating, conception, fertility and gestation indices were analyzed by the Fisher exact probability test (alpha = 0.05) with Bonferroni's correction. Evaluation of the neonatal sex ratio on postnatal day 1 was performed by the binomial distribution test (alpha = 0.05). Gender was determined for pups found dead on postnatal day 0 and these data were included in sex ratio calculations. Survival indices, post-implantation loss, and other incidence data among neonates were analyzed using the litter as the experimental unit by the censored Wilcoxon test (alpha = 0.05) as modified by Haseman and Hoel with Bonferroni's correction. Nonpregnant females were excluded from gestation and lactation body weights and body weight gains, feed consumption and organ weights. Both the Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control to keep the experiment-wise error rate at 0.05. Both were reported at the experiment-wise alpha level. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values

2. Indices:
i) The following reproductive indices were calculated from breeding and parturition records of animals in the study:
- Female mating index = (No. females with evidence of mating/No. paired) x 100
- Male mating index = (No. males with evidence of mating/No. paired) x 100
- Female conception index = (No. females with evidence of delivering a litter/No. mated) x 100
- Male conception index = (No. males siring a litter/No. mated) x 100
- Female fertility index = (No. females with evidence of delivering a litter/No. paired) x 100
- Male fertility index = (No. males siring a litter/No. paired) x 100
- Gestation index = (No. females delivering a viable litter/No. females delivering a litter) x 100
- Gestation survival index = percentage of delivered pups alive at birth
- Post-implantation loss = (No. implants – No. viable offspring)/(No. implants) x 100

ii) The following viability indices were calculated from lactation records of litters in the study:
- Day 1 or 4 pup survival index = (No. viable pups on day 1 or 4/No. born live) x 100
- Day 7, 14, or 21 pup survival index = (No. viable pups on day 7, 14 or 21/No. live after culling) x 100.

3. **Historical Control Data:** Not provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality:

a) P_1 generation: One male in the control group was found dead on day 93; cause of death was determined to be due to a perforated ulcer and associated secondary effects. In the 50 mg/kg bw/day group, one male was found dead on day 23; cause of death was undetermined. One female in the 1000 mg/kg bw/day group was found dead on day 69 due to respiratory distress secondary to choke syndrome (placental tissue in the esophagus).
These deaths were not considered to be related to treatment.
All remaining animals survived the duration of the study period.

b) P_2 generation: One male in the 250 mg/kg bw/day was found dead 6 days prior to the start of the pre-breeding phase; cause of death was undetermined.
This death was not considered to be related to treatment.
All remaining animals survived the duration of the study period.

2. Clinical Signs: There were no overt clinical signs of treatment-related toxicity in the P_1 and P_2 generation parent animals.

3. Body Weight and Body Weight Gain:

i) **P_1 generation**:

Premating: Refer to Table 2. For males, there was no treatment-related effect on body weight gain at any dose level tested.
For females, mean final body weight and body weight gain were slightly lower in the 1000 mg/kg bw/day group. However, final body weight fell within 5% of the control group value, findings were not statistically significant and there were no other effects on body weight or body weight gain noted for males or females in either the P_1 or P_2 generations. Hence, this finding was not considered to be treatment-related.

TABLE 2 - Body Weight and Food Consumption - Pre-mating[a]

Study week	Dose Group (mg/kg bw/day)			
	0	50	250	1000
P_1 Generation Males - Pre-mating				
Mean body weight (g)±SD Week 0	193.7±9.0	193.7±9.1	193.8±9.3	193.9±9.4
Week 5	401.7±34.9	392.3±30.2	402.9±35.5	397.9±25.5
Week 10 (% of control)	494.6±44.8 ---	481.5±41.1 (97.4%)	488.2±50.3 (98.7%)	485.8±31.4 (98.2%)
Mean body weight gain (g) Weeks 0-5[b]	208	198.6	209.1	204
Weeks 5-10[b]	92.9	89.2	294.4	87.9
Weeks 0-10[b] (% of control)	300.9 ---	287.8 (95.6%)	294.4 (97.8%)	291.9 (97.0%)
Mean food consumption, wks 0-10[b] g/animal/day (% of control)	26.27 ---	25.59 (97.4%)	26.11 (99.4%)	27.18 (103.5%)
P_1 Generation Females - Pre-mating				
Mean body weight (g)±SD Week 0	147.6±8.2	147.7±8.1	147.6±8.0	147.8±8.1
Week 5	238.5±18.6	228.5±23.8	235.0±22.0	230.1±20.1
Week 10 (% of control)	273.2±21.8 ---	267.0±27.6 (97.7%)	269.9±26.0 (98.8%)	259.4±24.8 (94.9%)
Mean body weight gain (g) Weeks 0-5[b]	90.9	80.8	87.4	82.3
Weeks 5-10[b]	34.7	38.5	34.9	29.3
Weeks 0-10[b] (% of control)	125.6 ---	119.3 (95.0%)	122.3 (97.4%)	111.6 (88.9%)
Mean food consumption, wks 0-10[b] g/animal/day (% of control)	18.04 ---	17.64 (97.8%)	17.96 (99.6%)	17.81 (98.7%)

[a] Data extracted from pages 71 to 74 and 81 to 84 of the study report; n=29/30.
[b] Calculated by the PMRA reviewer; standard deviations not available

Gestation: Refer to Table 3. There were no treatment-related findings.

Lactation: Refer to Table 3. There were no treatment-related findings.

TABLE 3 - Body Weight and Food Consumption - Gestation and Lactation[a]

Study week	Dose Group (mg/kg bw/day)			
	0	50	250	1000
P_1 Generation Females - Gestation				
Mean body weight (g)±SD Day 0 (% of control)	271.5±23.0 ---	271.6±30.5 (100.0%)	271.7±26.4 (100.1%)	258.8±26.1 (95.3%)
Day 21 (% of control)	412.5±31.4 ---	413.4±36.9 (100.2%)	413.1±36.9 (100.1%)	398.5±35.0 (96.6%)
Mean body weight gain (g)±SD Days 0-21 (% of control)	141.0±17.4 ---	141.7±21.1 (100.5%)	141.4±19.3 (100.3%)	138.3±13.7 (98.1%)
Mean food consumption, g/dam/day Days 0-21[b]: (% of control)	22.67 ---	22.63 (99.8%)	23.0 (101.5%)	23.3 (102.8%)
P_1 Generation Females - Lactation				
	0	50	250	1000
Mean body weight (g)±SD Day 0 (% of control)	304.1±23.9 ---	307.2±29.6 (101.0%)	306.3±24.5 (100.7%)	291.4±27.2 (95.8%)
Day 21 (% of control)	328.8±22.2 ---	321.4±26.3 (97.7%)	322.1±22.3 (98.0%)	314.3±21.5 (95.6%)
Mean body weight gain (g)±SD Days 0-21	24.7±13.7	14.2±14.7*	16.5±15.4	22.9±14.4
Mean food consumption, g/dam/day Days 0-21[b]: (% of control)	52.16 ---	50.53 (96.9%)	51.97 (99.2%)	54.87 (105.2%)

[a] Data extracted from pages 74, 75 and 84 to 87 of the study report; n=22 to 26.
[b] Calculated by the PMRA reviewer; standard deviations not available
* Statistically significantly different from control, $p<0.05$.

Post-mating (males): Refer to Table 4. There were no treatment-related findings.

TABLE 4 - Body Weight and Food Consumption - Post-mating, males[a]

Study week	Dose Group (mg/kg bw/day)			
	0	50	250	1000
Mean body weight (g)±SD Week 0 (% of control)	504.2±49.3 ---	492.7±40.5 (97.7%)	500.3±51.8 (99.2%)	500.0±31.2 (99.2%)
Week 6 (% of control)	559.9±51.7 ---	553.2±48.5 (98.8%)	557.3±60.1 (99.5%)	559.6±39.5 (99.9%)
Mean body weight gain (g) Weeks 0-6[b] (% of control)	55.7 ---	60.5 (108.6%)	57.0 (102.3%)	59.6 (107.0%)

Mean food consumption, g/dam/day Weeks 0-6[b]: (% of control)	26.48 ---	26.15 (98.8%)	26.55 (100.3%)	27.78 (104.9%)

[a] Data extracted from pages 71, 72, 81 and 82 of the study report; n=29/30.
[b] Calculated by the PMRA reviewer; standard deviations not available

ii) P_2 generation:

Premating: Refer to Table 5. There were no treatment-related findings.

TABLE 5 - Body Weight and Food Consumption - Pre-mating[a]

Study week	Dose Group (mg/kg bw/day)			
	0	50	250	1000
P_2 Generation Males - Pre-mating				
Mean body weight (g)±SD Week 0	119.2±21.0	117.9±17.9	118.2±15.6	117.8±12.8
Week 5	381.0±37.4	382.2±33.4	386.8±33.7	381.6±26.7
Week 10 (% of control)	505.3±50.5 ---	503.0±50.3 (99.5%)	508.5±52.7 (100.6%)	498.3±39.8 (98.6%)
Mean body weight gain (g) Weeks 0-5[b]	261.8	264.3	390.3	380.5
Weeks 5-10[b]	124.3	120.8	121.7	116.7
Weeks 0-10[b] (% of control)	386.1 ---	385.1 (99.7%)	390.3 (101.1%)	380.5 (98.5%)
Mean food consumption, wks 0-10[b] g/animal/day (% of control)	27.45 ---	28.07 (102.3%)	27.73 (101.0%)	28.29 (103.1%)
P_2 Generation Females - Pre-mating				
Mean body weight (g)±SD Week 0	103.6±15.2	103.2±14.4	103.8±12.2	105.6±10.6
Week 5	229.1±19.1	233.9±21.6	235.9±21.6	234.3±19.4
Week 10 (% of control)	277.4±25.2 ---	286.7±27.7 (103.4%)	281.3±25.9 (101.4%)	279.0±25.9 (100.6%)
Mean body weight gain (g) Weeks 0-5[b]	125.5	130.7	132.1	128.7
Weeks 5-10[b]	48.3	52.8	45.4	44.7
Weeks 0-10[b] (% of control)	173.8 ---	183.5 (105.6%)	177.5 (102.1%)	173.4 (99.8%)
Mean food consumption, wks 0-10[b] g/animal/day (% of control)	19.05 ---	19.50 (102.4%)	19.64 (103.1%)	19.79 (103.9%)

[a] Data extracted from pages 76 to 78 and 88 to 90 of the study report; n=27-30.
[b] Calculated by the PMRA reviewer; standard deviations not available

Gestation: Refer to Table 6. There were no treatment-related findings.

Lactation: Refer to Table 6. There were no treatment-related findings.

TABLE 6 - Body Weight and Food Consumption - Gestation and Lactation[a]

Study week	Dose Group (mg/kg bw/day)			
	0	50	250	1000
P$_1$ Generation Females - Gestation				
Mean body weight (g)±SD Day 0	275.5±22.7	285.4±29.0	281.5±27.4	275.0±22.5
Day 21 (% of control)	418.1±41.2 ---	425.4±40.0 (101.7%)	418.9±36.2 (100.2%)	417.8±38.8 (99.9%)
Mean body weight gain (g)±SD Days 0-21 (% of control)	142.6±26.3 ---	140.0±20.8 (98.2%)	137.3±22.4 (96.3%)	142.8±27.5 (100.1%)
Mean food consumption, g/dam/day Days 0-21[b]: (% of control)	23.63 ---	23.53 (99.6%)	23.77 (100.6%)	24.33 (103.0%)
P$_1$ Generation Females - Lactation				
	0	50	250	1000
Mean body weight (g)±SD Day 0	312.6±27.5	318.3±37.0	315.9±25.7	312.6±26.7
Day 21 (% of control)	337.0±23.5 ---	345.1±30.1 (102.4%)	338.7±24.4 (100.5%)	333.6±25.2 (99.0%)
Mean body weight gain (g)±SD Days 0-21	24.4±17.8 ---	25.3±16.4 (102.5%)	22.6±14.7 (92.6%)	21.0±11.3 (86.1%)
Mean food consumption, g/dam/day Days 0-21[b]: (% of control)	55.56 ---	55.09 (99.2%)	57.14 (102.8%)	56.10 (101.0%)

[a] Data extracted from pages 79, 80 and 91 to 94 of the study report; n=22 to 27.
[b] Calculated by the PMRA reviewer; standard deviations not available
* Statistically significantly different from control, $p<0.05$.

Post-mating (males): Refer to Table 7. There were no treatment-related findings.

TABLE 7 - Body Weight and Food Consumption - Post-mating, males[a]

Study week	Dose Group (mg/kg bw/day)			
	0	50	250	1000
Mean body weight (g)±SD Week 0 (% of control)	532.3±53.1 ---	529.1±53.5 (99.4%)	539.4±52.4 (101.3%)	519.8±43.1 (97.7%)
Week 7 (% of control)	607.2±67.1 ---	607.3±57.7 (100.0%)	612.8±66.4 (100.9%)	592.2±55.9 (97.5%)
Mean body weight gain (g) Weeks 0-7[b] (% of control)	74.9 ---	78.2 (104.4%)	73.4 (98.0%)	72.4 (96.7%)

Mean food consumption, g/dam/day Weeks 0-6[a]: (% of control)[b]	29.49 ---	30.24 (102.5%)	29.96 (101.6%)	30.16 (102.3%)

[a] Data extracted from pages 77 and 89 of the study report; n=29/30.
[b] Calculated by the PMRA reviewer; standard deviations not available

4. Food Consumption: P_1 and P_2 generations: Refer to Tables 2 to 7. There were no treatment-related effects on food consumption for either generation.

5. Test Substance Intake: Based on food consumption and body weight, the doses expressed as mean daily mg test substance/kg body weight during the 10 week-pre-mating period for the P_1 and P_2 generations are presented in Table 8. The values for the P_2 generation are considered to be representative of the test substance intake for the entire study.

TABLE 8 - Mean test substance intake during premating (mg/kg body weight/day)

	Male			Female		
	50	250	1000	50	250	1000
P_1	54.0±5.4	271±29	1093±127	51.9±3.2	260±18	1037±65
P_2	52.0±10.8	259±53	1030±232	49.3±8.0	245±41	973±167

6. Reproductive Function:

a. Estrous Cycle Length and Periodicity: There were no treatment-related effects on estrous cyclicity in the P_1 and P_2 generations.

b. Sperm Measures: There were no treatment-related effects on sperm count, sperm motility or sperm morphology in the P_1 and P_2 generations.

c. Sexual Maturation: There were no treatment-related effects on age at vaginal opening or age at preputial separation noted in the F_1 pups. (Not measured in the F_2 pups, since there were no findings observed in the F_1 pups).

7. Reproductive Performance:

i) **P_1 generation:** Refer to Table 9. There were no treatment-related findings.

TABLE 9 - Reproductive Performance[a], P_1 Parents

Observation	Dose Group (mg/kg bw/day)			
	0	50	250	1000
Number of females mated	30	30	30	30
Time to mating, days	2.9±2.4	2.8±2.3	2.5±1.	2.8±1.2
Gestation length, days	21.6±0.5	21.8±0.5	21.8±0.5	21.8±0.4
Gestation Index, %	100.0 (27/27)	100.0 (27/27)	96.7 (29/30)	100.0 (29/29)

Male Mating Index, %	96.7 (29/30)	96.6 (28/29)	100.0 (30/30)	100.0 (30/30)
Mating Index, %	96.7 (29/30)	96.7 29/30)	100.0 (30/30)	100.0 (30/30)
Male Conception Index, %	93.1 (27/29)	92.9 (26/28)	100.0 (30/20)	96.7 (29/30)
Female Conception Index, %	93.1 (27/29)	93.1 (27/29)	100.0 (30/30)	96.7 (29/30)
Male Fertility Index, %	90.0 (27/30)	89.7 (26/29)	100.0 (30/30)	96.7 (29/30)
Female Fertility Index, %	90.0 (27/30)	90.0 (27/30)	100.0 (30/30)	96.7 (29/30)
Post Implantation Loss	8.27±8.63	12.27±16.07	12.15±15.70	10.78±9.57

[a] Data extracted from pages 171 and 172 of the study report.

ii) P, generation: Refer to Table 10. There were no treatment-related findings.

TABLE 10 - Reproductive Performance[a], P, Parents

Observation	Dose Group (mg/kg bw/day)			
	0	50	250	1000
Number of females mated	30	30	30	30
Time to mating, days	3.1±2.2	3.3±2.3	3.1±2.4	2.9±1.7
Gestation length, days	21.8±0.5	21.9±0.4	21.9±0.5	21.9±0.3
Gestation Index, %	100.0 (25/25)	100.0 (23/23)	100.0 (28/28)	100.0 (27/27)
Male Mating Index, %	93.3 (28/30)	93.3 (28/30)	100.0 (29/29)	93.3 (28/30)
Mating Index, %	93.3 (28/30)	93.3 (28/30)	100.0 (30/30)	93.3 (28/30)
Male Conception Index, %	92.9 (26/28)	82.1 (23/28)	93.1 (27/29)	96.4 (27/28)
Female Conception Index, %	92.9 (26/28)	82.1 (23/28)	93.3 (28/30)	96.4 (27/28)
Male Fertility Index, %	86.7 (26/30)	76.7 (23/30)	93.1 (27/29)	90.0 (27/30)
Female Fertility Index, %	86.7 (26/30)	76.7 (23/30)	93.3 (28/30)	90.0 (27/30)
Post Inplantation Loss	8.35±9.04	7.96±7.62	9.16±10.25	7.88±7.65

[a] Data extracted from pages 173 and 174 of the study report.

8. Parental postmortem results

a) Organ Weights:

i) **P_1 generation:**

Males: Refer to Table 11. Full and/or empty cecal weights were increased in the 250 and 1000 mg/kg bw/day groups.

TABLE 11 - Mean Organ Weights, Males[a], absolute (g)±SD and relative (% bw)±SD

	Dose (mg/kg bw/day)			
Organ	0 (n=29)	50 (n=29)	250 (n=30)	1000 (n=30)
Cecum, full - absolute	5.223±1.131	4.951±0.940	6.493±1.584*	11.534±3.382*
- relative	1.001±0.228	0.961±0.197	1.251±0.327*	2.230±0.678*
Cecum, empty - absolute	2.206±0.320	2.172±0.333	2.418±0.427	3.022±0.437*
- relative	0.420±0.048	0.420±0.061	0.462±0.059*	0.580±0.070*

[a] Data obtained from page 97 in the study report.
* Statistically significantly different from control, $p < 0.05$
** Statistically significantly different from control, $p < 0.01$

Females: Refer to Table 12. Full and/or empty cecal weights were increased in the 250 and 1000 mg/kg bw/day groups.

TABLE 12 - Mean Organ Weights, Females[a], absolute (g)±SD and relative (% bw)±SD

	Dose (mg/kg bw/day)			
Organ	0 (n=27)	50 (n=27)	250 (n=29)	1000 (n=28)
Cecum, full - absolute	3.913±0.873	3.701±0.878	4.723±1.029*	6.653±1.667*
- relative	1.405±0.280	1.345±0.300	1.694±0.286*	2.469±0.537*
Cecum, empty - absolute	1.874±0.273	1.876±0.237	2.036±0.298	2.412±0.513*
- relative	0.675±0.092	0.682±0.067	0.739±0.080*	0.898±0.172*

[a] Data obtained from page 100 in the study report.
* Statistically significantly different from control, $p < 0.05$
** Statistically significantly different from control, $p < 0.01$

ii) **P_2 Generation:**

Males: Refer to Table 13. Full and/or empty cecal weights were increased in the 50, 250 and 1000 mg/kg bw/day groups.

TABLE 13 - Mean Organ Weights, Males[a], absolute (g)±SD and relative (% bw)±SD

	Dose (mg/kg bw/day)			
Organ	0 (n=30)	50 (n=29)	250 (n=29)	1000 (n=30)
Cecum, full - absolute	4.614±0.931	5.211±1.231	6.065±1.640*	8.700±1.948*
- relative	0.795±0.141	0.899±0.231	1.036±0.275*	1.546±0.375*
Cecum, empty - absolute	1.994±0.292	2.283±0.641	2.561±0.502*	2.878±0.382*
- relative	0.343±0.033	0.390±0.095*	0.439±0.097*	0.513±0.078*

[a] Data obtained from page 103 in the study report.
* Statistically significantly different from control, $p < 0.05$
** Statistically significantly different from control, $p < 0.01$

Females: Refer to Table 14. Full and/or empty cecal weights were increased in the 250 and 1000 mg/kg bw/day groups.

TABLE 14 - **Mean Organ Weights, Females**[a], absolute (g)±SD and relative (% bw)±SD

	Dose (mg/kg bw/day)			
Organ	0 (n=25)	50 (n=23)	250 (n=28)	1000 (n=27)
Cecum, full - absolute	3.493±0.793	3.753±0.936	4.093±1.123	5.050±1.287*
- relative	1.183±0.246	1.234±0.347	1.344±0.360	1.757±0.478*
Cecum, empty - absolute	1.590±0.245	1.580±0.237	1.744±0.330	2.059±0.513*
- relative	0.546±0.104	0.517±0.092	0.572±0.111	0.720±0.209*

[a] Data obtained from page 105 in the study report.
* Statistically significantly different from control, $p < 0.05$
** Statistically significantly different from control, $p < 0.01$

b) Pathology

1. Macroscopic Examination:

i) P_1 generation: Refer to Table 15. The only treatment-related finding was an increase in the size of the cecum, noted in the 1000 mg/kg bw/day group, both sexes, and in the 250 mg/kg bw/day group, females only.

Table 15 - Selected Gross Pathological Findings in the Cecum[a]

	Dose (mg/kg bw/day)			
Increased size	0	50	250	1000
Males	38015	38015	38015	21/30
Females	38015	38015	2/30	20/30

[a] Data obtained from page 36 in the study report.

ii) P_2 generation: Refer to Table 16. The only treatment-related finding was an increase in the size of the cecum, noted in the 250 and 1000 mg/kg bw/day groups, both sexes.

Table 16 - Selected Gross Pathological Findings in the Cecum[a]

	Dose (mg/kg bw/day)			
Increased size	0	50	250	1000
Males	38015	38015	2/30	17/30
Females	38015	38015	38106	38289

[a] Data obtained from page 37 in the study report.

2. Microscopic Examination:

P_1 and P_2 generations: There were no treatment-related findings.

B. OFFSPRING

1. Clinical Signs: There were no overt clinical signs of treatment-related toxicity in the F_1 or F_2 pups.

2. Viability:

a) P_1 litters: Refer to Table 17. There were no treatment-related findings.

TABLE 17 - Mean Litter Parameters[a]

Observation	Dose Group (mg/kg bw/day)			
	0 (n=27)	50 (n=27)	250 (n=29)	1000 (n=29)
# Stillborn	0.1±0.4	0.6±1.5	0.1±0.4	0.1±0.4
# Live pups at birth	13.3±2.4	12.9±3.6	12.9±3.2	12.9±1.9
# Live pups at day 4 (precull)	12.9±2.2	12.6±3.6	12.8±3.2	12.9±1.9
# Live pups at day 4 (postcull)	8.0±0.2	7.5±1.4	7.8±1.1	8.0±0.2
# Live pups at day 21	8.0±0.2	7.5±1.6	7.8±1.1	8.0±0.0
Survival Index, %, Day 1	98.1	98.3	99.5	99.2
Survival Index, %, Day 4	96.7	97.7	98.9	99.2
Survival Index, %, Day 7	100.0	99.5	100.0	100.0
Survival Index, %, Day 21	100.0	99.5	99.6	100.0
Gestation Survival Index, %	98.9	95.9	98.9	98.9
Post-implantation loss	8.27±8.63	12.27±16.07	12.15±15.70	10.78±9.57
Sex ratio (% males, day 1)	52	50	52	50

[b] Calculated by the PMRA reviewer; standard deviations not available.
[a] Data extracted from pages 171, 172 and 175 of the study report.

b) P_2 litters: Refer to Table 18. There were no treatment-related findings.

TABLE 18 - Mean Litter Parameters[a]

Observation	Dose Group (mg/kg bw/day)			
	0 (n=25)	50 (n=23)	250 (n=28)	1000 (n=27)
# Stillborn	0.2±0.4	0.2±0.4	0.2±0.5	0.2±0.5
# Live pups at birth	12.9±3.1	12.7±3.1	13.1±3.0	12.8±3.6
# Live pups at day 4 (precull)	12.8±3.2	12.0±4.1	12.7±3.2	12.6±3.5
# Live pups at day 4 (postcull)	7.8±1.0	7.5±1.8	7.7±1.5	7.7±0.9
# Live pups at day 21	7.8±1.0	7.5±1.8	7.6±1.5	7.4±1.4
Survival Index, %, Day 1	99.4	96.2	97.3	99.1
Survival Index, %, Day 4	99.1	94.5	96.7	98.6
Survival Index, %, Day 7	100.0	99.4	99.1	96.5
Survival Index, %, Day 21	100.0	99.4	99.1	96.5
Gestation Survival Index, %	98.5	98.6	98.7	98.3
Post-implantation loss	8.35±9.04	7.96±7.62	9.16±10.25	7.88±7.65
Sex ratio (% males, day 1)	52	56	48	51

[b] Calculated by the PMRA reviewer; standard deviations not available.
[a] Data extracted from pages 173, 174 and 176 of the study report.

3. Body Weight: Refer to Table 19.

a) F_1 and F_2 pups: There were no treatment-related findings.

TABLE 19 - Selected Mean Pup Body Weights (g)±SD, F_1 and F_2 litters[a]

Observation	Dose Group (mg/kg bw/day)			
	0	50	250	1000
F_1 pups				
Day 1: Male	7.1±0.7	7.1±0.8	7.3±0.6	7.3±0.5
Female	6.8±0.7	6.8±0.8	7.0±0.7	7.0±0.6
Day 7: Male	15.6±2.0	16.4±1.7	16.6±1.7	16.6±1.2
Female	15.1±0.9	15.9±1.9	16.0±1.5	16.0±1.1
Day 21: Male	50.8±4.8	51.2±6.0	50.7±4.4	53.0±4.2
Female	49.6±3.7	50.1±5.1	49.4±4.1	50.9±4.2
F_2 pups				
Day 1: Male	7.2±0.6	7.4±0.9	7.2±0.7	7.6±0.8
Female	6.8±0.6	6.9±1.0	6.9±0.6	7.1±0.7
Day 7: Male	16.0±1.9	16.9±1.6	16.6±1.6	17.4±1.8
Female	15.3±1.7	16.1±1.2	16.0±1.4	16.5±1.5*
Day 21: Male	54.2±5.7	53.6±4.3	54.1±4.6	54.5±4.6
Female	51.6±4.8	50.8±2.9	52.0±3.5	52.0±4.0

[a] Data extracted from pages 177 and 178 of the study report
* Statistically significantly different from control, $p<0.05$.
[b] Calculated by the PMRA reviewer; standard deviations not available.

4. Offspring Postmortem Results:

a) Organ Weights: There were no findings considered to be related to treatment in the F_1 or F_2 litters.

b) Pathology

1) Macroscopic Examination: There were no treatment-related effects for pups in the F_1 or F_2 litters.

2) Microscopic Examination: Microscopic examination was not conducted on offspring in the F_1 or F_2 litters.

III. DISCUSSION

A. Investigators' Conclusions: "Body weights of both P_1 and P_2 animals and weanling F_1 and F_2 pups were unaffected by treatment. Furthermore, there were no treatment-related effects on organ weights with the exception of the cecum. Statistically significant increases in absolute and relative full and empty cecal weights were identified in the 1000 mg/kg/day P_1 and P_2 adults of both sexes. Similar effect were seen at 250 mg/kg/day, although of lesser severity. At 50 mg/kg/day, the only significant parental effect was a statistically significant increase in relative empty cecal weights in P_2 males. Increased cecal size also was noted at gross necropsy of the 250 and 1000 mg/kg/day adults. There were no treatment-related histopathologic changes in any tissue examined, including the cecum. Thus, cecal enlargement was interpreted to represent an adaptive process to a physiological effect localized to the cecum and was not considered toxicologically significant. There were no adverse effects of XDE-750 on any parameter of reproductive function, pup survival, growth or development. Based upon the absence of any significant adverse effects, the No-Observed-Adverse-Effect-Level (NOAEL) for parental toxicity was considered to be 1000 mg/kg/day, while 1000 mg/kg/day (the highest dose level tested) was considered a No-Observed-

Effect-Level (NOEL) for reproductive toxicity."

B. Reviewer's Discussion: A two-generation reproduction study was conducted using CD (Crl:CD (SD) IGS BR rats, fed test diets containing technical XDE-750, purity 94.5%, at dietary concentrations of 0, 50, 250 or 1000 mg/kg bw/day (equal to 0, 52.0, 259 or 1030 mg/kg bw/day for males, and 0, 49.3, 245 or 973 mg/kg bw/day for females), continuously throughout the study period, 30 rats per sex per group. Each female in each generation was mated to produce one litter.

For parent animals, there were no treatment-related effects on mortality, clinical signs, body weight and body weight gain, food intake, reproductive function, reproductive parameters or histopathology. Full and/or empty cecal weights were increased in the P_1 generation, in the 250 and 1000 mg/kg bw/day groups, both sexes. In the P_2 generation, full and empty cecal weights were increased in the 1000 mg/kg bw/day group, both sexes, and in the 50 and 250 mg/kg bw/day groups, males only. At gross necropsy, cecal size was increased in the P_1 and P_2 generations, in the 250 and 1000 mg/kg bw/day groups, both sexes. In the absence of any histopathological findings in the ceca, and in the absence of any other treatment-related parental findings, the cecal findings were considered to be adaptive changes and were not considered to be adverse.

For pups, there were no treatment-related effects on clinical signs, viability/litter parameters, pup body weight and body weight gain, organ weights or gross pathology.

Based on the results obtained from this study, the NOAEL for parental toxicity was 1000 mg/kg bw/day (1030/973 mg/kg bw/day). The LOAEL could not be determined since there were no adverse, treatment-related effects noted at any dose level tested.

For reproductive toxicity, the NOAEL was 1000 mg/kg bw/day (1030/973 mg/kg bw/day). The LOAEL could not be determined since there were no treatment-related effects noted at any dose level tested.

For offspring toxicity, the NOAEL was 1000 mg/kg bw/day (1030/973 mg/kg bw/day). The LOAEL could not be determined since there were no treatment-related effects noted at any dose level tested.

C. Study Deficiencies: No scientific deficiencies were noted in the study.

005100

(18)

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** Steve Ary for
Fungicide/Herbicide Toxicological Evaluation
Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C)
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: *(Bacterial system, Salmonella typhimurium; E. coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100; OECD 471 (formerly OECD 471 & 472).

PC CODE: 005100 **DP BARCODE:** D305671

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750

CITATION: Mecchi, M.S., (2001) *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with XDE-750. Covanee Laboratories Inc., Vienna, Virginia. Covance Study No. 22338-0-422OECD, Dow Study ID 011058, October 16, 2001; Amendment Dates: November 8, 2001, February 19, 2004.Unpublished.

SPONSOR: The Dow Chemical Company, Midland, Michigan for Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a reverse gene mutation assay (MRID 46235636) in bacteria, strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* and strain WP2*uvr*A of *Escherichia coli* were exposed to XDE-750, purity 94.5%, dissolved in dimethylsulfoxide at concentrations of 0, 100, 333, 1000, 3300 or 5000 µg/plate in the presence and absence of an Aroclor 1254-stimulated rat liver metabolic activation system using the preincubation test. The results of the initial mutagenicity assay were confirmed in a second, independently conducted assay.

Cytotoxicity was not observed, nor was a precipitate formed at any dose level tested. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of treatment-induced mutant colonies above background levels.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-750
   - **Description:** Technical; beige-light orange powder.
   - **Lot/Batch #:** F-0031-143; TSN102319
   - **Purity:** 94.5% a.i.
   - **CAS #:** 150114-71-9
   - **Solvent Used:** Dimethylsulfoxide (DMSO)

2. **Control Materials:**
   - **Negative:** NA
   - **Solvent (final conc'n):** 50 µL/plate DMSO
   - **Positive:** Nonactivation:
     Sodium azide 2.0 µg/plate TA100, TA1535
     2-Nitrofluorene 1.0 µg/plate TA98
     ICR-191 2.0 µg/plate TA1537
     Other (list): 4-nitroquinoline-N-oxide 0.4 µg/plate WP2*uvr*A
     Activation:
     2-Aminoanthracene (2-anthramine) 2.5 µg/plate TA100, TA1535, TA1537; 25 µg/plate WP2*uvr*A
     Benzo[a]pyrene 2.5 µg/plate TA98

3. **Activation**: S9 derived from

✓	induced	✓	Aroclor 1254	✓	Rat	✓	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

S9 mix composition:

Component	Amount
H_2O	0.70 mL
1M NaH_2PO_4, pH 7.4	0.10 mL
0.25M Glucose-6-phosphate	0.02 mL
0.10M NADP	0.04 mL
0.825M KCl/0.2M $MgCl_2$	0.04 mL
S9 Homogenate	0.01 mL
	1.00 mL

4. **Test organisms:** *S. typhimurium* and *E. Coli* strains

	TA97	✓	TA98	✓	TA100		TA102		TA104
✓	TA1535	✓	TA1537		TA1538	✓	WP2P uvrA		

Properly maintained? ☑ Yes ☐ No

Checked for appropriate genetic markers (*rfa* mutation, R factor)? ☑ Yes ☐ No

5. Test Compound Concentrations Used:

a) Range Finding Study: ±S9 - 6.67, 10, 33.3, 66.7, 100, 333, 667, 1000, 3330 or 5000 µg/plate.
Single plates were used per dose level, per condition, and per strain (*S. typhimurium* TA100 and *E. coli* WP2*uvr*A)

b) Mutagenicity Assay:
Initial and Confirmatory Assays:
Nonactivated and activated conditions: 100, 333, 1000, 3300 or 5000 µg/plate, all test strains.

All concentrations of the test article, the vehicle controls and the positive controls were plated in triplicate.

B. TEST PERFORMANCE

1. Type of *Salmonella* Assay:

_ standard plate test
✓ pre-incubation (20 minutes)
_ "Prival" modification *(i.e. azo-reduction method)*
_ spot test
_ other

2. Protocol: For the range finding study, only tester strains TA100 and WP2*uvr*A were utilized. All tester strains were used in the mutagenicity assays. For both procedures, 50 µL aliquot of the appropriate test material concentration, positive controls or solvent, 100 µL of the bacterial cell culture and 500 µL of S9 mix or phosphate buffer (for tests with or without metabolic activation, respectively) were added to culture tubes pre-heated to 37±2°C . The tubes were vortexed and then incubated at 37°C for 20±2 minutes. After the incubation period, 2 mL of molten selective top agar containing biotin/histidine or tryptophan were added to each tube, and the mixture was vortexed and then poured onto the surface of 25 mL of minimal bottom agar plates. After the overlay had solidified, the plates were inverted and incubated at 37°C for 52±4 hours. Positive control materials were plated using a 50 µL plating aliquot. At the end of the incubation period, the number of histidine-dependent colonies on each plate were counted by automated colony counter or by hand. For all plates, the bacterial background lawn was evaluated both macroscopically and microscopically (dissecting microscope) for indications of cytotoxicity and test article precipitates.

3. Statistical Analysis: Not conducted. For all replicate platings, the mean revertants per plate and the standard deviation were calculated.

4. Evaluation Criteria:

Assay Validity: An assay is considered valid if:
i) the genetic characteristics of the test strains have been confirmed;
ii) the appropriate number of bacteria are plated, i.e., the density of tester strain cultures should be greater than or equal to 0.5×10^9 bacteria per mL;
iii) the positive controls elicit positive responses;
iv) the tester strain cultures must exhibit a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions; and,

v) a minimum of 3 non-toxic concentrations are required to evaluate assay data.

Positive Response: a) Tester Strain TA100: A response is considered positive if there is a reproducible, two-fold increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control, and there is evidence of a dose response.
b) Tester Strains TA98, TA1535, TA1537 and WP2*uvr*A: A response is considered positive if there is a reproducible, three-fold increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control, and there is evidence of a dose response.

II. REPORTED RESULTS

A. Chemical analysis: Analysis of the dosing solutions indicated the solutions ranged from 97 to 106 % of the target levels.

B. Preliminary Cytotoxicity Assay: In both the presence and absence of S9 mix, cytotoxicity was not observed, nor was a precipitate formed at any dose level tested, i.e., up to and including 5000 µg/plate. Thus, the highest concentration chosen for mutagenicity testing was 5000 µg/plate for all tester strains.

C. Mutagenicity Assay: The findings are presented in the following table.

Mean Revertants Per Plate (N = 3 plates)													
		TA98		TA100		TA1535		TA1537		WP2*uvr*A		Background Lawn	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
		+S9											
Vehicle Control		31	29	98	113	13	17	8	10	23	17	normal	
XDE-750 µg/plate	100	30	29	111	98	9	14	9	8	19	16	normal	
	333	26	27	100	101	13	10	8	9	24	18	normal	
	1000	28	36	114	110	12	11	10	11	25	21	normal	
	3330	29	48	98	102	10	12	9	11	26	20	normal	
	5000	26	32	106	101	12	13	8	12	23	17	normal	
Positive Control		353	390	1000	1218	112	136	139	163	625	657	normal	
		-S9											
Vehicle Control		20	20	93	100	12	10	9	9	19	17	normal	
XDE-750 µg/plate	100	15	17	109	103	14	15	9	9	28	14	normal	
	333	14	29	101	89	11	12	7	10	20	16	normal	
	1000	21	20	106	98	13	11	6	8	21	16	normal	
	3330	14	20	92	92	12	13	6	11	22	16	normal	
	5000	17	17	94	93	16	16	4	10	23	17	normal	
Positive Control		355	365	1228	1270	893	848	3661	4203	451	461	normal	
data taken from Tables 3-6, pp 24-27 of Report													

Five doses of the test substance ranging from 100 to 5000 µg/plate were evaluated with and without S9 activation using *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2*uvr*A. For both the initial mutagenicity assay and the confirmatory assay, there was no increase in the number of revertants per plate at any dose level tested, whereas the positive controls elicited significant increases in the number of revertant colonies in their respective strains.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the conclusions of the investigators that under the conditions of this study, XDE-750 did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes.

B. **Study Deficiencies:** No scientific deficiencies were noted in the study.

PMRA Primary Reviewer: Steve Wong, Ph.D.
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: [signature]
Date Aug 31 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)
Signature: [signature]
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Ames gene mutation - *Salmonella typhimurium* and *Escherichia coli* gene mutation assay; OPPTS 870.5100; OECD 471 (formerly OECD 471 & 472).

PC CODE: 005100
005209

DP BARCODE: D305671

TEST MATERIAL (PURITY):

GF-871 (amino-3,6-dichloro-2-propanol: 1,1',1'-nitrilotris-) (aqueous formulation consisting of 41.3% XDE-750 triisopropanolammonium (TIPA) salt as the active ingredient)

SYNONYMS: XDE-750 triisopropanolammonium (TIPA), X677349

CITATION: Mecchi, MS, March 2, 2004. *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with GF-871. Covance Laboratories Inc. (Covance), Vienna, VA. Covance Study No. 25552-0-422OECD, Dow Study ID: 031150, MRID 46235637. Unpublished

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

In two *in vitro* gene mutation assays (initial and confirmatory)(MRID 46235637), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2*uvr*A were exposed to GF-871 (contain 41.3% XDE-750 TIPA salt). The dose levels tested were 0, 33.3, 100, 333, 1000, 3330, and 5000 µg (based on XDE-750 TIPA)/plate, with or without S9 metabolic activation. The S9 fraction was derived from Aroclor 1254-induced male rat livers. Appropriate positive controls were included in the assays. No cytotoxicity was seen up to 5000 µg XDE-750 TIPA per plate. No test article precipitate was observed on any of the plates in the presence or absence of S9 mix. In the main and confirmatory mutagenicity tests, exposure to the test material did not result in increases in the number of revertants in any of the bacterial strains, at any dose level in either the absence and in the presence of metabolic activation. All the positive control compounds produced the expected increase in the number of revertant colonies, demonstrating the sensitivity of the test system.

This study is acceptable and satisfies the guideline requirement for a microbial gene mutation study, DACO 4.5.4, OPPS 870.5100, OECD 471.

COMPLIANCE: Signed and dated GLP, QA, and Data Confidentiality statements were provided.

The study was conducted based on the following test guidelines: US EPA OPPTS 870.5100, August (1998), OECD Guideline 471, updated and adopted July 21, 1997.

STUDY DATE: October 24 2003 - January 26, 2004

I. MATERIALS AND METHODS

A. Materials:

1. **Test material:** GF-871
   - **Description:** translucent dark-orange liquid
   - **Lot/Batch #:** lot#173-162-1A
   - **Purity:** aqueous formulation containing 41.3 % XDE-750 TIPA
   - **Solvent used:** deionized water

2. **Control materials:**
   - **Solvent:** deionized water
   - **Positive:**
     - S9: sodium azide 2.0 µg/plate TA100, TA1535
       2-nitrofluorene 1.0 µg/plate TA98
       ICR-191 2.0 µg/plate TA1537
       4-nitroquinoline-N-oxide 0.4 WP2uvrA
     + S9: 2-aminoanthracene 25 µg/plate WP2uvrA
       benzo(a)pyrene 2.5 µg/plate TA98

3. **Activation:**

Liver microsomal enzymes (S9 homogenate) were purchased from Molecular Toxicology, Inc, Lot Nos. 1612 (39.86 mg of protein per mL), 1581 (35.4 mg of protein per mL) and 1615 (39.5 mg of protein per mL). The homogenate was derived from livers of Aroclor 1254-treated male Sprague Dawley rats (500 mg/kg bw administered intraperitoneally). The S9 mix components were: H_2O, 1M NaH_2PO_4/Na_2HPO_4, pH7.4, 0.25 M glucose-6-phosphate, 0.10M NADP, 0.825M Kcl/0.2M $MgCl_2$, and S9 homogenate.

4. **Test organisms:**

S. typhimurium strains TA98, TA100, TA1535, TA1537 were obtained from Dr. BN Ames, University of California, and *Escherichia coli* WP2*uvr*A was obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland.

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538	X	WP2*uvr*A		
Properly maintained ?									Yes
Checked for appropriate genetic markers (rfa mutation, R factor) ?									Yes

5. **Test compound concentrations used:**

Range-finding study: (1 plate per concentration- TA100 and WP2*uvr*A)
±S9: 2.76, 4.14, 13.8, 27.6, 41.4, 138, 276, 414, 1380, 2070 ug XDE-750 TIPA/plate

Main and confirmatory tests: (3 plates per concentration –TA98, TA100, TA1535, TA1537 and WP2*uvr*A)
±S9: 0, 33.3, 100, 333, 1000, 3300, and 5000 µg XDE-750 TIPA/plate.

B. Test performance

Two independent assays (main and confirmatory) with and without S9-mix were performed. Vehicle and positive controls were included in each test. Triplicate plates for each dose level were used.

1. **Statistical analysis**: Mean and standard deviation were computed.

2. **Evaluation criteria**:

The assay was considered valid if the following criteria were met:

1. The solvent control data were within the laboratory's normal control range for the spontaneous mutant frequency. Acceptable ranges are: TA98 - 8-60; TA100 - 60-240; TA1535 - 4-45; TA1537 - 2-25; WP2*uvr*A - 5-40. Historical control values from test laboratory were presented.
2. The positive control induced at least 3-fold increase in the mutation frequency over the mean value of the vehicle control for that strain.

The test article was classified to induce a positive response if the following effect was met:

1. For tester strain TA100: The test article produced at least a 2-fold concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control.
2. For tester strains TA98, TA1535, TA1537, and WP2*uvr*A: The test article induced at least a 3-fold concentration-related and reproducible increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control.

II. REPORTED RESULTS

A. Concentration analysis

Concentration analysis indicated a range of 93 to 103% of the target concentrations.

B. Preliminary cytotoxicity assay

A preliminary cytotoxicity test using tester strains TA100 and WP2*uvr*A indicated the absence of cytotoxicity in TA100 in the presence of S9 mix, or with WP2*uvr*A in the presence or absence of S9 activation at concentrations ranging from 2.76 to 2070 µg XDE-750 TIPA/plate. A decrease in the number of revertant colonies was observed with TA100 in the absence of S9 mix at the highest concentration tested (2070 µg XDE-750 TIPA/plate), although the bacterial background lawn was observed to be normal. This decrease in revertant counts was not reproduced in the subsequent mutagenicity assays up to a maximum concentration of 5000 µg of the active ingredient XDE-750 TIPA per plate. Therefore, the decrease in revertant colonies observed was likely due to normal variability and was not actually an indication of cytotoxicity. No test article precipitate was observed on any of the plates in the presence or absence of S9 mix.

C. Mutagenicity assays

In both assays, the test material did not cause an increase in the number of revertant colonies at any dose level with any of the tester strains either in the absence of or in the presence of S9 metabolic activation. All positive controls produced significant increases in the number of revertant colonies, demonstrating the sensitivity of the assay system for the detection of gene mutation in bacteria. The findings are summarized in the following table.

	TA98	TA100	TA1535	TA1537	WP2uvrA	TA98	TA100	TA1535	TA1537	WP2uvrA
	Main assay - # revertant colonies/plate ± SD (N = 3 plates)									
	- S9					+S9				
solvent control	14±2	101±14	11±5	9±4	11±2	20±4	123±9	10±2	9±3	17±9

positive control		381±50	1099±86	493±26	2905±444	390±43	328±36	1014±102	83±2	125±4	164±45
XDE-750 TIPA, µg/plate	33.3	10±2	105±4	10±4	6±1	12±3	14±7	105±5	9±3	9±1	21±5
	100	11±1	99±12	6±1	7±3	4±4	17±3	102±10	7±3	9±1	18±7
	333	9±4	93±14	9±2	8±1	6±4	20±3	107±14	10±5	8±5	12±3
	1000	11±2	83±8	14±3	8±3	9±2	15±8	109±15	8±4	7±6	11±3
	3330	9±2	90±15	9±3	10±4	10±2	21±4	93±1	7±5	11±1	14±2
	5000	10±3	82±17	9±2	10±3	11±1	21±9	114±7	9±6	10±4	12±3
		Confirmatory assay - # revertant colonies/plate ± SD (N = 3 plates)									
		- S9					+S9				
solvent control		14±7	99±9	17±3	10±5	20±4	21±4	105±8	14±3	9±2	25±2
positive control		386±65	1506±15	926±107	4295±142	199±60	232±68	859±59	97±3	135±15	261±115
XDE-750 TIPA, µg/plate	33.3	18±7	87±6	12±3	3±1	18±3	20±6	108±8	13±3	7±6	23±3
	100	13±2	105±5	13±2	7±4	19±4	15±5	112±14	12±3	8±3	22±3
	333	11±1	111±10	15±3	7±4	23±6	15±6	117±6	13±4	6±3	16±4
	1000	14±5	99±10	14±3	7±3	15±8	17±6	106±6	11±3	8±5	25±7
	3330	13±3	95±9	11±2	7±2	16±3	24±6	109±19	11±4	11±5	22±4
	5000	15±2	102±2	11±4	6±1	15±1	24±3	124±16	11±2	6±2	21±8
data taken from Tables 2-5, pages 24-27 of Report.											

III. AUTHORS' CONCLUSIONS:

"*The results of the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay indicate that under the conditions of this study, the test article, GF-871, did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9).*"

IV. REVIEWERS' COMMENTS:

The study was properly conducted and the study authors conclusions are acceptable.

DEC 19

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** per
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster ovary (CHO) cells; OPPTS 870.5300; OECD 476.

PC CODE: 005100 **DP BARCODE:** D305671

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750

CITATION: Linscombe, V.A., et al (2001). Evaluation of XDE-750 in the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011037, July 23, 2001. MRID 46235801. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay (MRID 46235801) at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus, Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to XDE-750, purity 94.5%, in dimethylsulfoxide (DMSO), at concentrations of 0, 31.25, 62.5, 125, 250, 500, 1000, 1500 or 2070 µg/mL both in the absence and in the presence of metabolic activation. A second assay was conducted, using concentrations of 0, 250, 500, 1000, 1500 or 2070 µg/mL both in the absence and in the presence of metabolic activation.

The treated cultures both in the presence and absence of S-9 mix exhibited little to no cytotoxicity at any concentration tested. The positive controls did induce the appropriate response. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description: Technical; tan powder.
Lot/Batch #: F-0031-143; TSN102319
Purity: 94.5% a.i.
CAS #: 150114-71-9
Solvent Used: Dimethylsulfoxide (DMSO)

2 **Control Materials:**

Solvent control (final conc'n): 1% DMSO
Positive control: Nonactivation: (concentrations/solvent): Ethyl methanesulfonate (EMS), 621 µg/mL.
Activation: (concentrations/solvent): 20-Methylcholanthrene (20-MCA), 4 µg/mL.

3 **Activation**: S9 derived from

✓	induced	✓	Aroclor 1254	✓	Rat	✓	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

S9 mix Composition:
Thawed S-9 was reconstituted at a final concentration of 10% (v/v) in a "mix". The mix consisted of the following co-factors:

10 mM $MgCl_2 \cdot 6H_2O$
5 mM Glucose-6-phosphate
4 mM Nicotinamide adenine dinucleotide phosphate
10 mM $CaCl_2$
30 mM KCl
50 mM Sodium phosphate

The reconstituted mix was added to the culture medium to obtain the desired final concentration of S-9 in the culture, i.e., 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4 **Test Cells:** mammalian cells in culture

	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
✓	Chinese hamster ovary (CHO) cells		list any others

Media: Ham's F-12 nutrient mix supplemented with 5% (v/v) heat-inactivated, dialyzed fetal bovine serum, penicillin G (100 units/mL), streptomycin sulfate (0.1 mg/mL), fungizone (25 µg/mL) and an additional 2 mM L-glutamine.

Properly maintained?	✓ Yes	No
Periodically checked for Mycoplasma contamination?	✓ Yes	No
Periodically checked for karyotype stability?	Yes	✓ Not stated.
Periodically "cleansed" against high spontaneous background?	Yes	✓ Not stated.

5 **Locus Examined:** Thymidine kinase (TK) ✓ Hypoxanthine-guanine-phospho-ribosyl transferase (HGPRT) Na^+/K^+ ATPase

Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)	✓	6-thioguanine (6-TG) 10 µM		
		trifluorothymidine (TFT)				

6. Test Compound Concentrations Used:

a) Preliminary Toxicity Assay: Nonactivated and activated conditions: 16.2, 32.4, 64.7, 129.4, 258.8, 517.5, 1035.0 or 2070.0 µg/mL.

b) Gene Mutation Assay:

Assay 1: Nonactivated and activated conditions: 31.25, 62.5, 125, 250, 500, 1000, 1500 or 2070 µg/mL.

Assay 2: Nonactivated and activated conditions: 250, 500, 1000, 1500 or 2070 µg/mL.

B. TEST PERFORMANCE

1. Cell Treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (nonactivated) 4 hours (activated).

b. After washing, cells were cultured for ~6-8 days (expression period) before cell selection.

c. After expression, $2x10^5$ cells/dish (10 dishes/ group) were cultured for ~6-10 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for ~6-10 days without selective agent to determine cloning efficiency.

2. Statistical Methods: The frequency of mutants per 10^6 clonable cells were statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency (MF) variance. The actual plate counts were assumed to follow a Poisson distribution, therefore the mean plate count was used as an estimate of variance. A linear trend test and lack of fit test were employed as omnibus tests to compare treated groups to the negative control. If there was a significant trend or a significant lack of fit, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control. The lack of fit test is just an indicator that further analysis is needed. An additional comparison of the positive control to the negative control was conducted using a linear contrast statement.

3. Evaluation Criteria:

Assay Validity: The assay is considered valid if the MF in positive controls is significantly higher than the negative controls. The MF in the negative controls should be within reasonable limits of the laboratory historical control values and literature values.

Positive Response: A response is considered positive if it induces a statistically significant, dose-related, reproducible increase in MF. The final interpretation will also take into consideration such factors as the MF and cloning efficiencies in the negative controls.

200 100

II. REPORTED RESULTS

A. Chemical analysis: The test material was soluble in DMSO. The pH and osmolality of treatment medium containing 2066 µg/mL of the test material equivalent fo 10 mM was determined. There was no appreciable change in the osmotic pressure; the pH was slightly altered, but the change was not expected to interfere with the assay.

B. Preliminary Cytotoxicity Assay: Refer to Table 1. Eight concentrations of XDE-750 ranging from 16.2 to 2070.0 µg/mL were tested. The treated cultures both in the presence and absence of S-9 mix exhibited little to no cytotoxicity with the relative cell survival (RCS) values ranging from 80.1% to 188.1% in the absence of S-9 mix and 22.0% to 194.3% in the presence of S-9 mix. Therefore, dose levels chosen for the gene mutation assay, in both the presence and absence of S-9 mix, ranged from 31.25 to 2070 µg/mL.

Table 1 - % Relative Survivals (%RCS)[a]

Dose, µg/mL	Without S-9	With S-9
0	100	100
16.2	110	167.2
32.4	115.2	161.5
64.7	109.4	194.3
129.4	86.1	106.2
258.8	211.6	161
517.5	111.9	109.3
1035	80.1	78.3
2070	188.1	22

[a] data obtained from page 19 in the study report.

C. Mutagenicity Assay:

Assay 1: Refer to Table 2. In both the absence or presence of S-9 mix, eight concentrations of XDE-750 ranging from 31.25 to 2070 µg/mL were tested. The treated cultures both in the presence and absence of S-9 mix exhibited little to no cytotoxicity with the relative cell survival (RCS) values ranging from 74.4% to 125.6% in the absence of S-9 mix and 51.9% to 150.0% in the presence of S-9 mix. No statistically significant increases in MF were observed at any dose level assessed either in the presence or absence of S-9 mix.

Table 2 - Relative Survivals and Mutation Frequencies[a]

Dose, µg/mL	Mutation Frequency[b]		Mutation Frequency[b]	
	Without S-9		With S-9	
	% RS	MF	% RS	MF
0	100	11.2	100	14.9

31.25	99.7	7.8	84.5	5.3
62.5	101.4	5.9	105	3.9
125	103.4	6.4	70.2	11.4
250	83.3	6.3	83.3	9.7
500	89.3	6.2	77.6	4.1
1000	96.2	9.8	82.2	4.1
1500	90.1	6.6	66.8	8.1
2070	111.2	14.7	56.2	15.1
Positive control[c]	45.4	691.5*	53.1	429.8*

* statistically significantly different from control, $p<0.05$
[a] data obtained from pages 20 and 22 in the study report.
[b] 6-TFT resistant mutants/10^6 viable cells
[c] Positive control without S-9: EMS, 621 µg/mL; positive control with S-9: 20-MCA, 4 µg/mL.
% RF - percent relative survival adjusted by post treatment cell counts
MF - mutant frequency

Assay 1: Refer to Table 3. In both the absence or presence of S-9 mix, five concentrations of XDE-750 ranging from 250 to 2070 µg/mL were tested. The treated cultures both in the presence and absence of S-9 mix exhibited little to no toxicity with the relative cell survival (RCS) values ranging from 75.6% to 124.2% in the absence of S-9 mix and 85.5% to 121.6% in the presence of S-9 mix. No statistically significant increases in MF were observed at any dose level assessed either in the presence or absence of S-9 mix.

Table 3 - Relative Survivals and Mutation Frequencies[a]

Dose, µg/mL	Mutation Frequency[b]		Mutation Frequency[b]	
	Without S-9		With S-9	
	% RS	MF	% RS	MF
0	100	5.3	100	6.1
250	106	3.6	88.5	6.6
500	92.1	6.7	118.7	6
1000	96.8	7.4	103.3	7.3
1500	86.7	3.7	87.9	6.6
2070	84.2	4.9	94	12.9
Positive control[c]	35.1	514.3*	83.8	624.5*

* statistically significantly different from control, $p<0.05$
[a] data obtained from pages 21 and 23 in the study report.
[b] 6-TFT resistant mutants/10^6 viable cells
[c] Positive control without S-9: EMS, 621 µg/mL; positive control with S-9: 20-MCA, 4 µg/mL.
% RF - percent relative survival adjusted by post treatment cell counts
MF - mutant frequency

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the conclusions of the investigators that "Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that XDE-750 did not induce a mutagenic response in the CHO/HGPRT gene mutation assay."

B. Study Deficiencies: No scientific deficiencies were noted in the study.

005100
(20)

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** Steve Day for
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:** KJB
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/6/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics (chromosomal aberration) OPPTS 870.5375; OECD 473.

PC CODE: 005100 **DP BARCODE:** D305671

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Linscombe, V.A., et al (2002). Evaluation of XDE-750 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011040, April 25, 2002. MRID 46235802. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (MRID 46235802) (chromosomal aberration), primary rat lymphocyte cultures were exposed to XDE-750 (purity 94.5%) in 1% dimethylsulfoxide, at concentrations of 0, 32.3, 64.7, 129.4, 258.8, 517.5, 1035 or 2070 µg/mL with and without metabolic (S9) activation (4 h treatment and 24 h cell harvest). A second assay was conducted at 0, 125, 250, 500, 750, 1000, 1400, 1700 or 2070 µg/mL in the absence of S9 activation and 0, 62.5, 125, 500, 1000 or 2070 µg/mL with S9 activation (24 h continuous treatment with cell harvest at end of treatment). A third assay to confirm the results noted in assay 2 in the absence of S9 activation was conducted at 400, 600, 800, 1000, 1200, 1400, 1600, 1700, 1800 or 2070 µg/mL (24 h continuous treatment with cell harvest at end of treatment). XDE-750 was tested up to cytotoxic concentrations. Mitotic indices (MI) were ~63-64 % of control for assay 1 at 2070 µg/mL with or without S9, <50 % of control at ≥1000 µg/mL without S9 and 84 % of control with S9 at 2070 µg/mL for assay 2, and <50 % at ≥1200 µg/mL without S9 for assay 3. There was a statistically significant increase in the percent of metaphases with aberrations (excluding gaps) in rat lymphocyte cultures treated with XDE-750 at 1000, 1400 and 1700 µg/mL, in the absence of S9 activation, for cells exposed to the test material for 24 h. The response was reproducible but was only observed at levels causing a ≥50 % reduction in MI. The predominant type of chromosome aberration was chromatid break. The findings suggested cytotoxicity. Additionally, the magnitude of the response was relatively weak with the frequencies of aberrant cells in XDE-750-treated cultures being only slightly greater than the upper end of the laboratory historical control data. Positive controls induced the appropriate response. **It is, therefore, concluded that XDE-750 is not a clastogenic agent in the presence of metabolic activation but induced a weak clastogenic effect only at cytotoxic levels with metabolic activation.**

This study is classified as acceptable. This study satisfies the guideline requirement for an *In vitro* mammalian cytogenetics (chromosomal aberration) assay (OPPTS 870.5375).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description: Technical; tan powder.
Lot/Batch #: F-0031-143; TSN102319
Purity: 94.5% a.i.
CAS #: 150114-71-9
Solvent Used: Dimethylsulfoxide (DMSO)

2 **Control Materials:**

Solvent control (final conc'n): 1% DMSO
Positive control Nonactivation (concentrations/solvent): Mitomycin C (MMC): 0.5 µg/mL (Assay 1); or 0.05 and 0.75 µg/mL (Assay 2).
Activation (concentrations/solvent): Cyclophosphamide monohydrate (CP): 4 and 6 µg/mL (Assay 1 and Assay 2).

3 **Activation**: S9 derived from:

✓	induced	✓	Aroclor 1254	✓	Rat	✓	Liver
	non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

S9 mix Composition:
Thawed S-9 was reconstituted at a final concentration of 10% (v/v) in a "mix". The mix consisted of the following co-factors:

10 mM $MgCl_2 \cdot 6H_2O$
5 mM Glucose-6-phosphate
4 mM Nicotinamide adenine dinucleotide phosphate
10 mM $CaCl_2$
30 mM KCl
50 mM Sodium phosphate

The reconstituted mix was added to the culture medium to obtain the desired final concentration of S-9 in the culture, i.e., 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4 **Test Cells:** mammalian cells in culture

V79 cells (Chinese hamster lung fibroblasts)
✓ Rat lymphocytes

Human lymphocytes

Chinese hamster ovary (CHO) cells

Media: Media: RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated, fetal bovine serum, penicillin G (100 u/mL), streptomycin sulfate (0.1 mg/mL), fungizone (25 µg/mL), 20 µg/mL PHA and an additional 2 mM L-glutamine.

Properly maintained?	☑ Yes	☐ No
Periodically checked for *Mycoplasma* contamination?	☐ Yes	☑ Not applicable.
Periodically checked for karyotype stability?	☐ Yes	☑ Not applicable.

Lymphocytes were recovered from blood samples of three rats (assay 1 and 2) and from 3 rats (assay 3). Cultures were initiated in RPMI media for 48 h prior to use.

5. Test Compound Concentrations Used:
Assay 1: Non-activated and activated conditions: 32.3, 64.7, 129.4, 258.8, 517.5, 1035 or 2070 µg/mL.
Assay 2: Non-activated conditions: 125, 250, 500, 750, 1000, 1400, 1700 or 2070 µg/mL.
Activated conditions: 62.5, 125, 500, 1000 or 2070 µg/mL.
Assay 3: Non-activated conditions: 400, 600, 800, 1000, 1200, 1400, 1600, 1700, 1800 or 2070 µg/mL.
Activated conditions: Not tested.

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Not conducted.

2. Cytogenetic Assay:

		Test Material	Solvent Control	Positive Control
a.	**Cell exposure time:**			
	Non-activated:	4 h (Assay 1) 24 h (Assays 2 and 3)	4 h 24 h	4 h
	Activated	4 h (All assays)	4 h	4 h
b.	**Spindle inhibition**			
	Inhibition used/concentration:	Colcemid, 0.2 µg/mL		
	Administration time:	3 hours (before cell harvest)		

		Test Material	Solvent Control	Positive Control
c.	**Cell harvest time after termination of treatment:**			
	Non-activated:	24 h	24 h	24 h
	Activated:	24 h	24 h	24 h

d. Details of Slide Preparation: The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides and stained in Giemsa. All slides were coded prior to evaluation.

e. Metaphase Analysis

No. of cells examined per dose: 200

Scored for structural?	✓ Yes	No
Scored for numerical?	✓ Yes - Polyploidy only.	No
Coded prior to analysis?	✓ Yes	No

f. Evaluation Criteria:

Assay Validity: The assay is considered valid if the chromosomal aberration frequency in the positive control cultures is significantly higher than the negative controls. The aberration frequency in the negative controls should be within reasonable limits of the laboratory historical control values.

Positive Response: A response is considered positive if it induces a significant, dose-related, reproducible increase in the frequency of cells with aberrations.

g. Statistical Analysis: The proportion of cells with aberrations (excluding gaps) was compared by the following statistical methods. At each dose level, data from the replicates were pooled. A two-way contingency table was constructed to analyze the frequencies of cytogenetic abnormalities. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the two global hypotheses of (1) no differences in average number of cells with aberrations among the dose groups, and (2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric was used for the doses in the statistical evaluation. If either statistic was found to be significant at $\alpha = 0.05$ versus a one-sided increasing alternative, pairwise tests (i.e., control vs. treatment) were performed at each dose level and evaluated at $\alpha = 0.05$ again versus a one-sided alternative. Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells was pooled across replicates for the analysis and evaluated at $\alpha = 0.05$. The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

II. REPORTED RESULTS

A. Chemical analysis:

Analytical determination on dosing solutions prepared for each assay indicated that the actual levels were within 86-90 (assay 1), 104-111 (assay 2), and 105-109 (assay 3) % of the target levels. There was no appreciable change in the osmotic pressure or the pH of treatment medium containing ~2088 µg/mL (equivalent to 10 mM) of the test material.

B. Assay 1:

a) Relative Mitotic Index (RMI) Determination: Refer to Table 1.
In the absence of S9 mix, the RMI varied from 63.9% at the highest concentration of 2070 µg/mL to 124.1% at the lowest concentration of 32.3 µg/mL. In the presence of S-9 mix, the RMI was 63.1% at 2070 µg/mL and 81.0% at 32.3 µg/mL. Based on these results, doses of 517.5, 1035 and 2070 µg/mL in the absence of S-9 mix, and doses of 64.7, 1035 and 2070 µg/mL in the presence of S-9 mix were chosen for analysis.

Table 1 - Relative Mitotic Indices (RMI) of Rat Lymphocytes after 4-Hour (±S9) Treatment with XDE-750[a]

Non-activated (-S9)		Activated (+S9)	
Dose (µg/mL)	(RMI%)	Dose (µg/mL)	(RMI%)
1% DMSO[b]	100	1% DMSO[b]	100
32.3	124.1	32.3	81
64.7	114.5	64.7	82.1
129.4	119.3	129.4	73.8

258.8	115.7	258.8	63.1
517.5	113.3	517.5	69
1035	92.6	1035	72.6
2070	63.9	2070	63.1
MMC, 0.5[c]	73.5	CP, 4.0[c]	61.9
		CP, 6.0[c]	59.5

[a] Data obtained from pages 27 and 28 in the study report.
[b] negative control
[c] positive control - MMC = mitomycin C; CP = cyclophosphamide

b) Chromosomal Aberration Analysis: Refer to Table 2.
Non-activated and activated systems: There was no increase in the incidence of polyploid cells at any dose level tested. There were no statistically or biologically significant increases in the percentage of aberrant cells, compared to the solvent control values, either in the absence or presence of S9 mix.

Table 2 - Number of Cells with Structural Aberrations/200 cells, excluding gaps[a]

Non-activated (-S9)		Activated (+S9)	
Dose (µg/mL)	Number of Aberrant Cells	Dose (µg/mL)	Number of Aberrant Cells
1% DMSO[b]	2 (1.0%)	1% DMSO[b]	1 (0.5%)
517.5	3 (1.5%)	64.7	1 (0.5%)
1035	2 (1.0%)	1035	1 (0.5%)
2070	3 (1.5%)	2070	2 (1.0%)
MMC, 0.5[c]	26 (20.8%)*	CP, 6.0[c]	33 (18.9%)*

[a] Data obtained from pages 30 and 31 in the study report.
[b] negative control
[c] positive control - MMC = mitomycin C; CP = cyclophosphamide
* statistically significantly different from control, $p < 0.05$

C. Assay 2:
a) Relative Mitotic Index (RMI) Determination: Refer to Table 3.
In the absence of S9, there was an increase in cytotoxicity with increasing concentration, i.e., the RMI ranged from 97.7% at the lowest concentration to 18.0% at the highest concentration and levels ≥1400 µg/mL had ≥ 50 % reduction in MI. In the presence of S-9 mix, there was little to no toxicity observed at any concentration tested. Based on these results, doses of 127, 750 and 1400 µg/mL in the absence of S-9, and doses of 62.5, 1000 and 2070 µg/mL in the presence of S-9 were chosen for analysis.

Table 3 - Relative Mitotic Indices (RMI) of Rat Lymphocytes after 24-Hour (-S9) or 4-Hour (+S9) Treatment with XDE-750[a]

Non-activated (-S9)		Activated (+S9)	
Dose (µg/mL)	(RMI%)	Dose (µg/mL)	(RMI%)
1% DMSO[b]	100	1% DMSO[b]	100

125	97.7	62.5	95
250	73.7	125	81.8
500	67.7	500	82.6
750	60.2	1000	81
1000	50.4	2070	84.3
1400	33.1		
1700	27.8		
2070	18		
MMC, 0.05[c]	45.9	CP, 4.0[c]	83.5
MMC, 0.075[c]	45.9	CP, 6.0[c]	71.9

[a] Data obtained from pages 32 and 33 in the study report.
[b] negative control
[c] positive control - MMC = mitomycin C; CP = cyclophosphamide

b) Chromosomal Aberration Analysis: Refer to Table 4.

Non-activated System: There was no increase in the incidence of polyploid cells at any dose level tested. A statistically significant increase in the number of cells with chromosome aberrations was observed at 1000, 1400 and 1700 µg/mL, compared to the solvent control value. However, the frequency of aberrant cells in the cultures treated with 1000 and 1400 µg/mL XDE-750 fell within the laboratory historical background range, whereas the frequency of aberrant cells in the cultures treated with 1700 µg/mL XDE-750 was slightly higher than the highest historical control value. Chromatid breaks were the only aberration observed at all test levels with the exception of a single chromatid exchange at 1700 µg/mL. (Historical control data obtained from studies carried out at the same laboratory, conducted between 1990 and 2001, indicated that the historical control range of values for % of cells with aberrations excluding gaps was 0.0% to 6.5%).

Activated System: There was no increase in the incidence of polyploid cells at any dose level tested. There were no statistically or biologically significant increases in the percentage of aberrant cells, compared to the solvent control value.

Table 4 - Cells with Structural Aberrations/200 cells, excluding gaps[a]

Non-activated (-S9)		Activated (+S9)	
Dose (µg/mL)	Aberrant Cells	Dose (µg/mL)	Aberrant Cells
1% DMSO[b]	1 (0.5%)	1% DMSO[b]	1 (0.5%)
125	2 (1.0%)	62.5	4 (2.0%)
750	2 (1.0%)	1000	2 (1.0%)
1000	9 (4.5%)*	2070	5 (2.5%)
1400	10 (5.0%)*		
1700	15 (7.5%)*		
MMC, 0.075[c]	34 (34.0%)*	CP, 6.0[c]	26 (17.3%)*

[a] Data obtained from pages 35 and 36 in the study report.

[b] negative control
[c] positive control - MMC = mitomycin C; CP = cyclophosphamide
* statistically significantly different from control, $p < 0.05$

D. Assay 3:

a) Relative Mitotic Index (RMI) Determination: Refer to Table 5.

In the absence of S9, the RMI varied from 20.3% at the highest concentration of 2070 µg/mL to 81.2% at the lowest concentration of 400 µg/mL. MI for levels ≥800 µg/mL were generally <50 %. Based on these results, doses of 1000, 1400 and 1700 µg/mL were chosen for analysis.

Table 5 - Relative Mitotic Indices (RMI) of Rat Lymphocytes, after 24-Hour (-S9) Treatment with XDE-750[a]

Non-activated (-S9)	
Dose (µg/mL)	**(RMI%)**
1% DMSO[b]	100
400	81.2
600	78.3
800	49.3
1000	47.8
1200	52.2
1400	37.7
1600	34.8
1700	29
1800	33.3
2070	20.3
MMC, 0.05[c]	58
MMC, 0.075[c]	50.7

[a] Data obtained from page 37 in the study report.
[b] negative control
[c] positive control - MMC = mitomycin C

b) Chromosomal Aberration Analysis: Refer to Table 6.

Non-activated and activated systems: There was no increase in the incidence of polyploid cells at any dose level tested. A statistically significant increase in the number of cells with chromosome aberrations was observed at 1400 and 1700 µg/mL, compared to the solvent control value, which were slightly higher than the highest historical control value. In agreement with non-activated data from assay 2, chromatid breaks were generally the only aberration seen. There were, however, three chromatid exchanges and one chromatid break at 1400 µg/mL and one chromatid exchange at 1700 µg/mL.

200

Table 6 - Cells with Structural Aberrations/200 cells, excluding gaps[a]

Non-activated (-S9)	
Dose (µg/mL)	**Aberrant Cells**
1% DMSO[b]	5 (2.5%)
1000	9 (4.5%)
1400	14 (7.0%)*
1700	15 (7.5%)*
MMC, 0.05[c]	22 (11.0%)*

[a] Data obtained from page 39 in the study report.
[b] negative control
[c] positive control - MMC = mitomycin C
* statistically significantly different from control, $p < 0.05$

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the conclusions of the investigators that XDE-750 induced a clastogenic response in rat lymphocyte cultures treated continuously for 24 hours. The response was reproducible but occurred at levels that caused a ~50 % decrease in MI and consisted primarily of chromatid breaks. The magnitude of the response was relatively weak with the frequencies of aberrant cells in test material treated cultures being only slightly greater than the upper end of the laboratory historical control data. We conclude, therefore, that XDE-750 induced chromosome aberration but only at cytotoxic concentrations; the clastogenic response was induced secondary to cytotoxicity.

B. **Study Deficiencies:** No scientific deficiencies were noted in the study.

201

005100 (21)

PMRA Primary Reviewer: Steve Wong, Ph.D. Signature: Steve Wong
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division Date Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. Signature: Karlyn
Registration Action Branch 2, Health Effects Division (7509C) Date 9/16/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics/chromosome aberration - rat lymphocytes; OPPTS 870.5375; OECD 473

PC CODE: 005100 005209 **DP BARCODE:** D305671

TEST MATERIAL (PURITY):

GF-871 (amino-3,6-dichloro-2-propanol: 1,1',1'-nitrilotris-) (aqueous formulation consisting of 41.3% XDE-750 TIPA salt as the active ingredient)

SYNONYMS: XDE-750 triisopropanolammonium (TIPA), X677349

CITATION: Linscombe, VA, KM Jackson, MR Schisler, February 27, 2004. Evaluation of GF-871 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID 031134, MRID 46235803. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

GF-871 (an aqueous formulation containing 41.3% XDE-750 TIPA) was evaluated in an *in vitro* chromosomal aberration assay using rat lymphocytes (MRID 46235803). Approximately 48 h after the initiation of whole blood cultures, cells were treated in the absence of S-9 for 4 or 24 h and for 4 h in the presence of S-9 activation with concentrations ranging from 0 (negative control) to 4000 µg XDE-750 TIPA per mL (limit dose of ~10 mM) of culture medium. Based on the mitotic indices, cultures treated for 4 h with targeted concentrations of 0, 1000, 2000, and 4000 µg/mL in the absence and presence of S-9 activation and cultures treated for 24 h without S9 at 0, 500, 1000, and 2000 µg/mL were assessed for incidence of chromosomal aberrations. S9 mix, purchased from Molecular Toxicology Inc, Boone, NC, was prepared form Aroclor-1254 treated male Sprague Dawley rats. There were no significant increases in the frequencies of cells with aberrations in the 4 h activation assay or the 24 h continuous treatment without activation. There was a statistically significant increase in the frequencies of cells with aberrations in the 4 h non-activation treatment at concentrations of 1000 and 4000 µg/mL treatment, but not at 2000 µg/mL (2.0%). The frequencies of aberrations at these two concentrations (2.5 and 3.0%, respectively) were not considered to be biologically significant since the aberration frequencies were within the laboratory historical negative control values and there was no dose response. The statistical finding was attributed to the chance occurrence of 0% aberrant cells in the negative controls. In a confirmatory assay, cultures were treated for 4 h in the absence of S-9 activation at targeted concentrations ranging from 0 to 4000 µg/mL. The incidence of chromosomal abnormalities was assessed from cultures treated at 0, 1000, 2000, and 4000 µg/mL. Statistical analyses of the data did not identify a significant difference between the negative control and any of the treated cultures. Cultures treated with the positive control chemicals (mitomycin C without S-9 and

207

cyclophosphamide with S-9) had significantly higher incidences of abnormal cells. Thus, GF-871 was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay with rat lymphocytes.

This study is classified as acceptable. This study satisfies the requirement for DACO 4.5.6, OPPTS 870.5375, OECD 473 for *in vitro* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, QA, and Data Confidentiality statements were provided. The study was conducted based on the following test guidelines: US EPA OPPTS 870.5375, 1998; OECD Guideline 473, 1997.

STUDY DATE: September 25, 2003 - February 13, 2004

I. MATERIALS AND METHODS

A. Materials:

1 **Test material:** GF-871
Description: brown liquid
Lot/Batch #: lot#173-162-1A, Ref# TSN104110
Purity: an aqueous formulation containing 41.3 % XDE-750 TIPA
CAS #: 150114-71-9, 000122-20-3

2. **Control materials:**
Vehicle: water/cell culture medium
Positive: - S-9: 4 h - 0.5 ug/mL mitomycin C (MC), 24 h - 0.05 and 0.075 ug/mL MC/treatment media
+ S-9: 4.0 and 6.0 ug/mL cyclophosphamide monohydrate (CP)/ treatment media

3. **Activation:**

Liver microsomal enzymes (S-9 homogenate) were purchased from Molecular Toxicology, Inc, Lot Nos 1612 (39.86 mg of protein per mL), 1581 (35.4 mg of protein per mL), and 1615 (39.5 mg of protein per mL). The homogenate was derived from livers of Aroclor 1254-treated male Sprague Dawley rats (500 mg/kg bw administered intraperitoneally).

4. **Test cells:**

Lymphocytes were obtained from male Sprague-Dawley rat (outbred Crl: CD BR, ~11 week-old). Cultures were initiated by inoculating approximately 0.5 mL of whole blood per 5 mL of culture medium. The cultures were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, antibiotics and antimycotics, 30 ug/mL phytohemagglutinin, and 2 mM L-glutatmine. Approximately 48 h after culture, lymphocytes were harvested.

5. **Test compound concentrations used:**

The dosing solutions were adjusted for purity of the active ingredient, XDE-750 TIPA. The following concentrations (based on XDE-750 TIPA) were tested:

Non-activated conditions: Assay A1- 4 h - 62.5, 125, 250, 500, 1000, 2000, 4000 ug/mL
Assay A1- 24 h - 31.3, 62.5, 125, 250, 500, 1000, 2000, 4000 ug/mL
Assay B1- 4 h - 62.5, 125, 250, 500, 1000, 2000, 4000 ug/mL
Activated conditions: Assay A1- 4 h - 62.5, 125, 250, 500, 1000, 2000, 4000 ug/mL

B. Test performance

Two independent assays (main and confirmatory) with and without S9-mix were performed. Vehicle and positive controls were included in each test. Duplicate plates for each dose level were used.

Approximately 48 h after initiation of the whole blood cultures, lymphocytes were harvested and dispensed into 15 mL sterile centrifuge tubes (~5.5 mL/tube, 2 cultures per dose level). The cells were exposed to medium (RPMI 1640, HEPES, and antibiotics) containing the test or positive or negative control substances for ~4 h at 37°C and the exposure was terminated by washing the cells with culture medium. The cells were then placed in individual sterile disposable tissue culture flasks along with ~4.5 mL of the original culture medium until the time of harvest. The cultures were harvested at approximately 24 h after treatment initiation (ie, ~20 h after treatment termination). A second set of cultures was treated with the test material continuously for 24 h (~1.5 normal cell cycle length). Stock solutions of the treatments were added directly to the culture flasks at 48 h after initiation of the cultures and these cultures were harvested 24 h later.

Colcemid, 0.2 µg/mL, was added approximately 3 h prior to harvest. The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa. All slides were coded prior to evaluation. Mitotic indices (MI) were determined as the number of cells in metaphase among 1000 cells/replicate and expressed as percentages. One hundred metaphases/replicate were examined, where possible, from coded slides at each selected concentration of the test chemical and the negative controls (200 cells/ treatment) for structural abnormalities. In the positive control cultures, 50-100 metaphases/replicate (100-200 cells/treatment) were examined for abnormalities. The microscopic coordinates of those metaphases containing aberrations were recorded. Only those metaphases that contained 42±2 centromeres were scored with the exception of cells with multiple aberrations, in which case accurate counts of the chromosomes were not always possible. Structural chromosomal abnormalities that were counted included chromatid and chromosome gaps, chromatid breaks and exchanges, chromosome breaks and exchanges, and miscellaneous (chromosomal disintegration, chromosomal pulverization, etc). Those cells with ≥5 aberrations/cell were classified as cells with multiple aberrations. Gaps were not included in calculations of total cytogenetic aberrations. In addition, 100 metaphases/replicate were examined for the incidence of polyploidy. The data were used to calculate the following parameters:

% Cells with aberrations = Aberrant cells (excluding cells with gaps) x 100 / # metaphases evaluated
Aberrations/100 cells = Total aberration (excluding gaps, miscellaneous and severely damaged) / # metaphases evaluated

3. **Statistical analysis**:

The proportion of cells with aberrations (excluding gaps) was compared by the following statistical methods. At each dose level, data from the replicates were pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the two global hypotheses of (1) no differences in average number of cells with aberrations among the dose groups, and (2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc) was used for the doses in the statistical evaluation. If either statistic was found to be significant at $\alpha = 0.05$ versus a one-sided increasing alternative, pair-wise tests (ie, control vs treatment) were performed at each dose level and evaluated at $\alpha = 0.05$ again versus a one-sided alternative. Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells was pooled across replicates for the analysis and evaluated at $\alpha = 0.05$. The data were analyzed separately based on the presence or absence of S-9 and on the exposure time.

4. **Evaluation criteria**:

For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the negative controls. The aberration frequency in the negative control should be within reasonable limits of the laboratory historical values (provided). A test chemical

is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

II. REPORTED RESULTS

A. Chemical analysis

The analytically detected concentrations of the test material in the stock solutions varied from 99.2 to 115% of the targets with the exception of the lowest concentration where the observed value was 58.1% of the target. Measurement of pH and osmolality indicated that neither parameters appreciably changed in medium containing 4000 µg/mL, equivalent to the limit dose of XDE-750 TIPA.

B. Preliminary cytotoxicity assay Not performed.

C. Mutagenicity assay Results are presented in the following table.

		Total aberrations / # cells with aberrations, excluding gaps, per 100 cells, based on duplicate cultures						Mean % mitotic index			
		Assay A1			Assay B1	historical controls		Assay A1			Assay B1
		-S9, 4h	+S9, 4h	-S9, 24h	-S9, 4h	-S9	+S9	-S9, 4h	+S9, 4h	-S9, 24h	-S9, 4h
solvent control		0.0/0.0	2.0/2.0	1.0/1.0	0.5/0.5	0.5-1.8	0.8-2.1	6.6	4.8	6.4	18.4
positive control		18/14[*a1]	38/30[*a2]	23/15[*a3]	41/37[*a1]			4.1[a1]	1.0[a2], 1.7[a4]	3.1[a3], 3.4[a5]	9.2[a1]
XDE-750 TIPA, µg/mL	31.3	-	-	-	-					7.2	
	62.5	-	-	-	-			5.0	5.3	7.1	18.1
	125	-	-	-	-			5.6	5.5	7.2	17.5
	250	-	-	-	-			5.1	5.8	6.5	17.4
	500	-	-	1.0/1.0	-			5.9	5.0	5.4	16.9
	1000	2.5/2.5*	0.0/0.0	2.5/2.5	1.0/1.0			5.6	4.1	4.0	15.0
	2000	2.0/2.0	3.0/3.0	2.5/2.5	3.0/3.0			7.2	4.6	2.4	13.4
	4000	3.0/3.0*	4.5/4.0	-	0.5/0.5			4.5	4.4		1.3

- not done; * significantly different from vehicle control, $\alpha = 0.05$;
positive controls: [a1] = MC, 0.5 µg/mL; [a2] = CP, 4 µg/mL; [a3] = MC, 0.075 µg/mL; [a4] = CP, 6 µg/mL; [a5] = MC, 0.05 µg/mL
historical values were mean ranges collected from 1994 to 2004 (11 studies)
data taken from Tables 3A-8 pages 23-32 of Report

In the initial assay, moderate toxicity was observed (32% reduction in MI) in cultures treated with GF-871 at 4000 µg/mL without metabolic activation (4 h treatment). The remaining cultures at the lower concentrations (62.5, 125, 250, 500, 1000, and 2000 µg/mL) had reductions in MI ranging from 0 to 24%. In the presence of S-9 activation, there was little to no evidence of cytotoxicity. Reductions in MI ranged from 0 to 15%. Cultures treated for 24 h without S-9 activation had reductions in MI of 38, 63 and 80% at concentrations of 1000, 2000, and 4000 µg/mL, respectively, while the remaining cultures had reductions in MI ranging from 0 to 16%. Based upon these results, cultures treated with 1000, 2000, and 4000 µg/mL GF-871 for 4 h (with and without S-9) and cultures treated with 500, 1000, and 2000 µg/mL for 24 h (without S-9) were chosen for the determination of chromosomal aberration and polyploidy. Among the cultures treated with the positive control chemicals for 4 h, MC (0.5 µg/mL) and CP (4 µg/mL) were selected for evaluation of aberration in the absence and presence of S-9, respectively. Cultures treated with 0.075 µg/mL MC were selected for evaluation to serve as the

positive control for the 24 h in the absence of S-9. There were no significant increases in the incidence of polyploid cells in GF-871-treated cultures as compared to the vehicle control values. In the 4 h non-activated assay, the frequency of cells with aberration in the vehicle control was 0% and the corresponding values at treatment levels 1000, 2000, and 4000 µg/mL were 2.5, 2.0, and 3.0%, respectively. Statistical analyses of these data identified a significant trend test and significant differences between the vehicle control and 1000 and 4000 µg/mL, but not 2000 µg/mL treatments. The frequency of aberrant cells at 1000 and 4000 µg/mL (2.5 and 3.0%, respectively) was identified as statistically different from the concurrent vehicle value (0% aberrant cells). However, the aberrant cell frequencies observed in the treated cultures were within the laboratory historical control values. Hence, it was concluded that the statistically identified differences was of little biological significance and the result of the chance occurrence of 0% aberrant cells among the vehicle control samples. In the activation assay, cultures treated with GF-871 at 1000, 2000, and 4000 µg/mL had aberrant cell frequencies of 0, 3.0, and 4.0%, respectively as compared to the vehicle control value of 2.0%. There were no statistically significant differences in aberrant cells between cultures treated with GF-871 for 4 h in the presence of S-9 activation and the corresponding vehicle control; all values were within the laboratory historical background range. A second assay with GF-871-treatment of cultures in the presence of S-9 was not considered necessary since the results of the initial test yielded clearly negative results. In the non-activated assay, where cultures were treated continuously for 24 h with GF-871, the frequencies of aberrant cells at 500, 1000, and 2000 µg/mL were 1.0, 2.5, and 2.5%, respectively, as compared to the vehicle control value of 1.0%. There were no statistically significant differences between GF-871-treated and vehicle control values and all values were within the laboratory historical background range. Significant increases in the frequency of cells with aberration were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MC (without S-9, 4 h treatment), CP (with S-9) and MC (without S-9, 24 h treatment) cultures were 14%, 30%, and 15.3%, respectively.

In the repeat assay, to verify the statistical finding in initial assay (without S-9, 4 h), rat lymphocyte cultures were treated with GF-871 for 4 h in the absence of S-9 at 62.5, 125, 250, 500, 1000, 2000, and 4000 µg/mL and cells were harvested 20 h after treatment termination. There was little to moderate cytotoxicity evident as determined by reductions in MI ranging from 1.6 to 27.2%. Based on these results, concentrations of 1000, 2000 and 4000 µg/mL were selected for the determination of chromosomal aberration and of polyploidy. There were no significant increases in the incidence of polyploid cells in GF-871-treated cultures as compared to the vehicle control. The frequency of cells with aberration in the vehicle control was 0.5% and the corresponding values at treatment levels 1000, 2000 and 4000 µg/mL were 1.0, 3.0, and 0.5%, respectively. Statistical analyses of these data did not identify significant differences between the vehicle control and any of the GF-871-treated cultures. The frequencies of aberrant cells observed in GF-871-treated cultures were within the laboratory historical background range. Significant increases in the frequency of cells with aberration were observed in cultures treated with the positive control chemical. The aberrant cell frequency in MC treated cultures was 37.0%. The results from the initial assay, 4 h without S-9, were interpreted not to be biologically significant since the aberration frequencies in the GF-871-treated cultures were within the laboratory historical negative control values and were not reproducible in this repeat assay. The results of this repeat assay confirmed that the statistical finding in the initial assay was attributable to the chance occurrence of 0% aberrant cells in the vehicle controls and hence of no biological relevance.

III. AUTHORS' CONCLUSIONS:

"Thus, GF-871 was considered to be non-genotoxic in this in vitro chromosomal aberration assay utilizing rat lymphocytes."

IV. REVIEWERS' COMMENTS:

The study was properly conducted and the authors conclusions are acceptable.

V. DEFICIENCIES: There were no deficiencies.

005100
(12)

PMRA Primary Reviewer: Steve Wong, Ph.D.
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: [signature]
Date [illegible]

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)
Signature: [signature]
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* gene mutation assay in Chinese hamster ovary cells; OPPTS 870.5300; OECD 476.

PC CODE: 005100
005209

DP BARCODE: D305671

TEST MATERIAL (PURITY):

GF-871 (amino-3,6-dichloro-2-propanol: 1,1',1'-nitrilotris-) (aqueous formulation consisting of 41.3% XDE-750 TIPA salt as the active ingredient)

SYNONYMS: XDE-750 triisopropanolammonium (TIPA), X677349

CITATION: Linscombe, VA, MR Schisler, SD Seidel, February 25, 2004. Evaluation of GF-871 in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) Forward mutation assay. Toxicology & Environmental Research and MRID 46235804. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

GF-871 (an aqueous formulation containing 41.3% XDE-750 TIPA) was evaluated in an *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay (MRID 46235804). The genotoxic potential of GF-871 was assessed in the absence and presence S9 activation at 250, 500, 1000, 2000, or 4000 µg XDE-750 TIPA /mL (limit dose of approximately 10 mM). The adequacy of the experimental protocol for detection of induced mutation was confirmed by positive controls, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9. Vehicle control cultures were treated with the solvent used to dissolve the test material. The results indicated acceptable cell survival (85.7-131.4 %) in cultures treated with GF-871. The mutation frequencies (MF) observed in cultures treated with GF-871 at any concentration in the absence and presence of S9 were similar to the concurrent vehicle control values. All MF were within a reasonable range of historical background values. The positive control chemicals in the absence and presence of S9 induced significant increases in MF verifying the adequacy of the test protocol. Thus, it was concluded that GF-871 did not induce a gene mutation response in the assay system employed.

This study is classified as acceptable. This study satisfies the guideline requirement for an *in vitro* mammalian cell gene mutation study, DACO 4.5.5., OPPTS 870.5300, and OECD 476.

COMPLIANCE: Signed and dated GLP, QA, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** GF-871
   - **Description:** brown liquid
   - **Lot/Batch #:** Lot#173-162-1A, Ref# TSN104110
   - **Purity:** aqueous formulation containing 41.3 % XDE-750 TIPA
   - **CAS #:** 150114-71-9, 000122-20-3

2. **Control materials:**
   - **Solvent control (conc'n):** water, 1%
   - **Positive control:** -S9: 621 ug/mL ethyl methanesulfonate (EMS) in culture media
     +S9: 4.0 ug/mL 20-methylcholanthrene (20-MCA) in dimethylsulfoxide (DMSO)

3. **Activation**

Microsomal enzymes (S9 homogenate), derived from livers of Aroclor 1254-treated male Sprague Dawley rat, were purchased from Molecular Toxicology, Inc, Boone, NC. Thawed S9 was reconstituted at a final concentration of 10% (v/v) in a "mix". The mix consisted of the following cofactors:10 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM $CaCl_2$, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, ie, 2% v/v. Hence, the final concentration of the cofactors in the culture medium is 1/5 of the concentrations stated above.

4. **Test cells:** mammalian cells in culture

	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
x	Chinese hamster ovary (CHO) cells		list any others

Media: Ham's F-12 nutrient mix supplemented with 5% heat-inactivated dialyzed fetal bovine serum, antibiotics and antimycotics, and an additional 2 mM L-glutamine

Properly maintained?	x	Yes		No
Periodically checked for Mycoplasma contamination?	x	Yes		No
Periodically checked for karyotype stability?		Yes	x	No
Periodically "cleansed" against high spontaneous background?	x	Yes		No

The cell line CHO-K1-BH4, originally obtained from Dr. Abraham Hsie, Oak Ridge National Laboratory, Oak Ridge, TN, was used in this study. Stock cultures were stored at about -80°C or below. The cultures were periodically checked for mycoplasma contamination (American Type Culture Collection, Manassas, VA). The cells were grown as monolayers in plastic disposable tissue culture labware under standard conditions of ~5% CO_2 in air at 37°C in a humidified incubator.

5. **Locus Examined:** **thymidine kinase (TK)** x **hypoxanthine-guanine-phospho-ribosyl transferase (HGPRT)** **Na^+/K^+ ATPase**

Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)	x	6-thioguanine (6-TG)		
		trifluorothymidine (TFT)				

6. **Test compound concentrations used:**

-S9: Preliminary cytotoxicity test - 31.3, 62.5, 125, 250, 500, 1000, 2000, 4000 ug XDE-750 TIPA/mL
Mutagenicity assay - 250, 500, 1000, 2000, 4000 XDE-750 TIPA/mL

+S9: Preliminary cytotoxicity test - 31.3, 62.5, 125, 250, 500, 1000, 2000, 4000 ug XDE-750 TIPA/mL
Mutagenicity assay - 250, 500, 1000, 2000, 4000 XDE-750 TIPA/mL

B. TEST PERFORMANCE

1. **Cell treatment:**

a. Cells were exposed to GF-871, vehicle, or positive controls for 4 h (±S9).

b. After washing, cells were cultured for 6-8 days (expression period) before cell selection.

c. After expression, $2x10^5$ cells/dish (10 dishes/group) were cultured for 6-10 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 6-8 days without selective agent to determine cloning efficiency.

2. **Statistical methods:**

The frequencies of mutants per 10^6 clonable cells were statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the MF variance. The actual plate counts were assumed to follow a Poisson distribution; therefore the mean plate count was used as an estimate of variance. If the analysis of variance is significant at $\alpha = 0.5$, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control ($\alpha = 0.05$, one-sided). An additional comparison of the positive control to the negative control ($\alpha = 0.05$) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant

3. **Evaluation criteria:**

For an assay to be acceptable, the MF in positive controls should be significantly higher than the vehicle controls, and the MF in the vehicle controls should be within reasonable limits of the laboratory historical control and literature values. The test chemical was considered positive if it induced a statistically significant, dose related, reproducible increase in MF. The final interpretation of the data took into consideration such factors as the MF and cloning efficiencies in the vehicle controls.

II. REPORTED RESULTS

A. Chemical analyses

The analytically detected concentrations of the test material in the stock solutions varied from 99.2 to 115% of the target with the exception of the lowest concentration where the observed value was 58.1% of the target. The pH and osmolality of treatment medium containing approximately 4000 µg XDE-750 TIPA/mL did not show appreciable differences from culture medium with the solvent alone.

B. Preliminary cytotoxicity assay

In a preliminary toxicity assay, the test material was assayed at concentrations of 31.3, 62.5, 125, 250, 500, 1000, 2000, and 4000 µg/mL in the absence and presence of S9 activation. The highest level tested was the limit dose of ~10 mM. The treated cultures both with and without S9 activation showed

little to no cytotoxicity with the relative cell survival (RCS) values ranging from 87.5 to 126.0% in the absence of S9 and 76.8 to 100.2% in the presence of S9. Based upon the results of this assay, concentrations of 250 to 4000 µg/mL of the test material were selected for the gene mutation assay in both the presence and absence of metabolic activation.

C. **Mutagenicity assay** The results are summarized in Table 1.

The test material concentrations tested in this assay, both with and without S9 activation, were 250, 500, 1000, 2000, and 4000 µg/mL. Based on RCS values (88.5-121.8% without S9, 85.7-131.4% with S9), little to no cytotoxicity was observed. The MF observed in cultures treated with the test material in the absence and presence of S9 were not significantly different from the concurrent vehicle control values at any concentration. All MF were within a reasonable range of historical background values.

The positive control chemicals with and without S9 induced significant increases in MF and these data confirmed the adequacy of the test protocol for detecting induced mutations.

Table 1: Relative cell survival (RCS) and mutation frequencies (MF) in cultures treated with GF-871 (mean of 3 dishes; no SD presented in original Report).

		-S9			+S9		
		% RCS	MF/10^6 cells	historical MF	% RCS	MF/10^6 cells	historical MF
vehicle control (1% distilled water)		76.2	5.8	2.5-9.6 (10 studies from 1993-2003)	105.6	7.3	3.2-9.7 (10 studies from 1993-2003)
		123.8	6.1		94.4	4.6	
GF-871 µg/mL (based on XDE-750 TIPA)	250	110.7	10.2		100.4	8.0	
		95.2	9.8		100.0	9.9	
	500	93.7	1.4		131.4	6.9	
		94.4	7.3		95.7	7.4	
	1000	88.5	11.5		86.3	10.1	
		103.8	5.9		102.5	13.6	
	2000	94.8	9.2		85.7	11.2	
		105.9	9.0		127.6	9.0	
	4000	121.8	7.6		98.7	2.8	
		103.1	6.5		114.8	2.5	
positive controls (-S9 = EMS, 621 µg/mL; +S9 = MCA, 4 µg/mL)		36.8	396.8*		83.6	200.9*	
		31.8	316.1*		81.2	187.5*	

RCS = (mean # of colonies/dish in the treated/mean # of colonies /dish in negative control) x 100; * $p<0.05$
data taken from Tables 2-3, pp 20-21 of Report

III. AUTHORS' CONCLUSIONS:

"Based upon the frequency of TG' mutants recovered in cultures treated with the test material, it was concluded that GF-871 did not induce a mutagenic response in the CHO/HGPRT gene mutation assay."

IV. REVIEWER'S COMMENTS:

The study was properly conducted and the study authors' conclusions are acceptable.

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:** for
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Aug 31, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C) **Date** 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice OPPTS 870.5395; OECD 474.

PC CODE: 005100 **DP BARCODE:** D305671

TEST MATERIAL (PURITY): XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Spencer, P.J. and T.A. Gorski (2002). Evaluation of XDE-750 in the Mouse Bone Marrow Micronucleus Test. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID 011125, March 7, 2002. MRID 46235805. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a mouse bone marrow micronucleus assay (MRID 46235805), 6 male CD-1 (ICR)BR mice/dose were dosed once daily fro 2 consecutive days by oral gavage with XDE-750, purity 94.5%, at dose levels of 0 (vehicle control), 500, 1000 or 2000 mg/kg bw. Bone marrow cells were harvested from mice at 24 hours post-treatment. The vehicle was 0.5% Methocel.

There was no treatment-related mortality, nor clinical signs of toxicity. The positive control induced the appropriate response. **There was no treatment-related increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any dose level tested, after any treatment time. It is, therefore, concluded that XDE-750 did not induce a clastogenic effect in either sex at any sacrifice time.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** XDE-750

Description: Technical: tan powder.
Lot/Batch #: F-0031-143; TSN102319
Purity: 94.5% a.i.
CAS #: 150114-71-9
Vehicle Used: 0.5% Methocel.

2 **Control Materials:**

		Final Volume:	Route:
Vehicle:	0.5% Methocel	10 mL/kg bw	Oral gavage
Positive control:	Cyclophosphamide-monohydrate	10 mL/kg bw Final Dose: 120 mg/kg bw	Oral gavage

3 **Test Animals:**

Species:	Male mice.
Strain:	CD-1(ICR)BR
Age/weight at study initiation:	8 weeks of age; 28.3 g to 37.1 g.
Source:	Charles River Laboratories, Portage, Michigan.
No. animals used per dose	**Range-finding test:** 4/sex/dose. **Micronucleus test:** 6 males/dose; an additional 6 in high dose group (possible replacements in the event of deaths occurring during the observation period).
Properly Maintained?	Yes

4 **Test Compound Administration:**

	Dose Levels (mg/kg bw)	Final Volume	Route
Preliminary:	0, 500, 1000 or 2000	0, 50, 100 and 200 mg/mL	Oral
Main Study:	0, 500, 1000 or 2000	0, 50, 100 and 200 mg/mL	Oral

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

a. Test Compound:

Dosing:		once	✓	twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	✓	24 hr		48 hr		72 hr
Other:										

b. Vehicle Control:

Dosing:		once	✓	twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	✓	24 hr		48 hr		72 hr
Other:										

c. Positive Control:

Dosing:	✓	once		twice (24 hrs apart)		Other

Other:												

2 **Tissues and Cells Examined:**

Bone marrow:	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE: more mature RBCs) examined per animal:	200
Other: NA	

3. Details of Slide Preparation: At 24 hours after dosing, animals were sacrificed by CO_2 asphyxiation. The distal end of the femur was severed and bone marrow was aspirated with a 25-gauge needle into a 3 mL syringe containing 0.5 mL fetal bovine serum. Contents of the syringe were transferred into a 1.5 mL centrifuge tube containing 0.5 mL of serum. After centrifugation and re-suspension, Wedge smears were prepared on microscope slides, air-dried, stained with Wright-Giemsa using an automatic slide stainer. The slides were coded prior to scoring.

4. Evaluation Criteria:
Assay Validity: The assay is considered valid if the range of MN-PCE values in the negative controls are within reasonable limits of the recent (past 5 years) laboratory background range; there is a significant increase in the incidence of MN-PCE in the positive control treatment as compared to the concurrent negative controls; and the mean percent PCE is greater than 5% in one or more of the test material treated groups.

Positive Response: A response is considered positive if there is a statistically significant dose-related increase in MN-PCE frequency at one or more dose levels accompanied by a dose response.

Negative Response: A response is considered negative if there is no statistically significant dose-related increase in MN-PCE as compared to the negative control; the MN-PCE values in the treated animals are within reasonable limits of the recent (within 5 years) laboratory historical control range; and the mean percent PCE values in one or more of the test material treated groups is greater than 5%.

5. Statistical Methods: The raw data on the counts of MN-PCE for each animal were first transformed by adding one to each count and then taking the natural log of the adjusted number. The transformed MN-PCE data and the data on percent PCE were analyzed separately by a one-way analysis of variance. Pairwise comparisons of treated vs. control groups were done, if the dose effect was significant, by Dunnett's t-test, one-sided (upper) for MN-PCE and two-sided for the percent PCE. Linear dose-related trend tests were performed only if any of the pairwise comparisons yielded significant differences. The alpha level at which all tests were conducted was 0.05.

II. REPORTED RESULTS

A. Chemical Analysis: Dosing solutions were prepared once before dosing on day 1 and the concentration of the test material in the dosing solutions was verified using HPLC/UV. Results showed that nominal concentrations ranged from 96 to 104 % of the target levels.

B. Preliminary Toxicity Assay: There were no mortalities, no changes in body temperature nor any treatment-related clinical observations noted at any dose level tested. Since there were no appreciable differences in toxicological response of males and females to the test material, only males were evaluated in the main study. The limit dose of 2000 mg/kg bw/day was selected for the micronucleus test based

upon the lack of toxicity.

C. Micronucleus Assay:

1. Toxicity: All animals survived, and there were no clinical signs of treatment-related toxicity.

2. Micronucleus Assay: Refer to Table 1. There were no statistically or biologically significant increases in the incidence of MN-PCEs, or in the percentage of PCEs, over the vehicle control values, at either sampling time. The positive control induced statistically and biologically significant increases in the incidence of MN-PCEs and PCEs at 24 hours.

Table 1 - Mean # of Micronucleated Polychromatic Erythrocytes in Male Rats[a], at 28 hours

Dose, mg/kg bw	Total # PCEs scored[b]	Mean percent PCE	Mean incidence MPCE[c]
Vehicle control	2000	70.6±2.6	3.9±1.7
500	2000	66.0±6.7	2.3±0.9
1000	2000	63.9±4.6	1.9±1.3
2000	2000	70.4±4.4	2.8±1.6
Positive control, CPA	2000	46.5±11.1*	75.4±24.1*

[a]data obtained from page 43 in the study report.
[b] total # PCEs examined per animal; 6 males/group
[c]Mean incidence of micronucleated polychromatic erythrocytes per 1000 PCEs.
** statistically significantly different from control, $p<0.05$.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the conclusions of the investigators that the test material, XDE-750, did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes when given as a single oral dose once daily for 2 consecutive days up to the limit dose of 2000 mg/kg/day to male CD-1 mice. Hence, under the experimental conditions used XDE-750 is considered negative in this test system.

B. Study Deficiencies: No scientific deficiencies were noted in the study.

(23)

PMRA Primary Reviewer: Steve Wong, Ph.D.
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: [signature]
Date 24 Jan 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)
Signature: [signature]
Date 9/4/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian bone marrow chromosome aberration - mouse micronucleus assay OPPTS 870.5385; OECD 473

PC CODE: 005100 005209 **DP BARCODE:** D305671

TEST MATERIAL (PURITY):

GF-871 (amino-3,6-dichloro-2-propanol: 1,1',1'-nitrilotris-) (aqueous formulation consisting of 41.3% XDE-750 TIPA salt as the active ingredient)

SYNONYMS: XDE-750 triisopropanolammonium (TIPA), X677349

CITATION: Spencer, PJ, VA Linscombe, and J Grundy, February 25, 2004. Evaluation of GR-871 in the mouse bone marrow micronucleus test. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID 031136, MRID 46235806. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, IN, USA.

EXECUTIVE SUMMARY:

The *in vivo* genotoxic potential of GF-871 (a formulation containing 41.3% XDE-750 TIPA) was evaluated by examining the incidence of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow (MRID 46235806). The test material was administered to male CD-1 mice, 6/group, by oral gavage at 0 (negative control), 500, 1000, or 2000 (limit dose) mg XDE-750 TIPA/kg bw/d for 2 days. The highest dose level of 2000 mg/kg bw was based upon the results of a range-finding test where at this dose, no treatment-related deaths, toxicity or changes in body temperature were observed in male or female mice. The mice were sacrificed 24 h after dosing. Bone marrow cells from both femurs were collected and evaluated. A total of 2000 polychromatic erythrocytes from each mouse was examined for micronucleus (MN) formation. The proportion of PCE among erythrocytes was determined based upon 200 erythrocytes per animal and the results expressed as a percentage. Mice treated orally with 120 mg/kg bw cyclophosphamide monohydrate and sacrificed 24 h later served as positive controls. All animals survived to the end of the observation period with no treatment-related signs of toxicity. There were no statistically significant increases in the frequencies of MN-PCE in groups treated with GF-871 as compared to the negative controls. The positive control mice showed a significant increase in the frequency of MN-PCE as compared to the negative control animals. There were no statistically significant differences in the percent PCE in groups treated with the test material as compared to negative controls, while the positive control group showed a significant decrease in the relative proportion of PCE among erythrocytes. Under the experimental conditions used, GF-871 was considered to be negative in the mouse bone marrow micronucleus test.

This study is acceptable and satisfies the guideline requirement for an *in vivo* mammalian bone marrow chromosome aberration - mouse micronucleus assay OPPTS 870.5385; OECD 473.

COMPLIANCE:

Signed and dated GLP, QA, and Data Confidentiality statements were provided.
The assay was conducted to meet the U.S. EPA OPPTS 870.5395 (1998) and OECD (1997) test guidelines.

STUDY DATE: October 28, 2003 - February 8, 2004.

I. MATERIALS AND METHODS

A. Materials:

1 **Test material:**

Description:	brown liquid
Lot/Batch #:	Lot#173-162-1A, Ref# TSN104110
Purity:	aqueous solution containing 41.3 % XDE-750 TIPA
CAS #:	150114-71-9, 000122-20-3

2 **Control materials:**

Vehicle:	water, concentration 10 mL/kg bw
Positive:	cyclophosphamide monohydrate in distilled water, 120 mg/kg bw

3 **Test animals:**

Species:	mouse	
Strain:	outbred CD-1(1CR)BR	
Age/weight at study initiation:	8 week old; 29.0-35.4 g	
Source:	Charles River Labs Inc, Portage, MI, USA	
Housing:	individually or 2/cage in stainless steel cages	
Diet:	LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St Louis, MO, USA) *ad libitum*	
Water:	municipal water *ad libitum*	
Environmental conditions:	**Temperature:**	no information
	Humidity:	no information
	Air changes:	12-15 exchanges per hour
	Photoperiod:	12 h dark/ 12h light (0600-1800 h)
Acclimation period:	7 days	

4. **Animal assignment/dose levels:**

The mice were randomized by a stratified randomization procedure using body weights and distributed into the various treatment groups noted in Table 1.

Table 1: Study design

	GF-871, mg/kg bw/d for 2 days				cyclophosphamide, mg/kg bw
	0 (vehicle)	500	1000	2000	120
N (number of ♂)	6	6	6	6	6

4. **Test procedures:**

Dose range-finding test:

This assay was conducted to aid the selection of dose levels for the micronucleus test. Groups of mice (4/sex/dose) were treated with 1000 and 2000 mg/kg bw /d of GF-871 (based on XDE-750 TIPA) for 2 days and observed for 72 h after dosing for any signs of toxicity. The relative body temperatures of the treated mice were monitored. A body temperature increase of 1°C or a decrease of >3°C for more than 5 h can confound interpretation of the study results since body temperature changes outside this range had been previously reported to cause an increase in micronucleus formation.

Micronucleus test:

Groups of male mice, 6/group, were administered by oral gavage GF-871 at 0, 500, 1000, and 2000 mg/kg bw/d (based on XDE-750 TIPA) on two consecutive days. The positive control substance, cyclophosphamide (CP), was orally administered only once at 120 mg/kg bw. The mice were observed daily for mortality and clinical signs of toxicity. Body weight of individual mouse was recorded prior to dosing and at scheduled sacrifice. Approximately 24 h after dosing, the mice were euthanized by CO_2 exposure and killed. Bone marrow samples were obtained from both femurs of each mouse and processed for examination of micronucleus.

Two thousand polychromatic erythrocytes (PCE) were examined from each mouse and the number of micronucleated PCE (MN-PCE) was recorded. Micronuclei were identified as darkly stained bodies with smooth contours and varying shapes such as round, almond, or ring. The ratio of PCE to NCE in the bone marrow was determined in the micronucleus test by examining 200 erythrocytes. The ratio was expressed as PCE x100/PCE+NCE.

3. Statistical analysis:

The raw data on the counts of MN-PCE for each animal were first transformed by adding one to each count and then taking the natural log of the adjusted number. The transformed MN-PCE data and the data on percent PCE were analyzed separately by a one-way analysis of variance. Pair-wise comparisons of treated vs control groups were done, if the dose effect was significant, by Dunnett's t-test, one-sided (upper) for MN-PCE and two-sided for the percent PCE. Linear dose-related trend tests were performed only if any of the pair-wise comparisons yielded significant differences. The α level at which all tests were conducted was 0.05.

4. Evaluation criteria:

A test was considered valid if all of the following conditions are met:
- The range of MN-PCE values in the negative controls are within reasonable limits of the recent (past five years) laboratory background range.
- There was a significant increase in the incidence of MN-PCE in the positive control treatment as compared to the concurrent negative controls.
- The mean for % PCE value in one or more of the test material treated groups was >20% of the control value indicating no undue effect on erythropoiesis (toxicity).

A test material was considered positive in this assay if the following was met:
- Statistically significant increase in MN-PCE frequency at one or more dose levels accompanied by a dose response.

A test material was considered negative in this assay if the following criteria were met:
- No statistically significant dose related increase in MN-PCE are compared to the negative control.

A test result not meeting the criteria for either for the positive or the negative response was considered to be equivocal.

II. REPORTED RESULTS

A. Chemical analysis

Dosing solutions were analyzed for actual concentrations and were found to be 122-140 % of the target doses.

B. Dose-range finding study

There were no mortalities. There were no appreciable effects on the body weights, clinical signs of toxicity, or changes in the body temperature of male and female mice following treatment with GF-871. Thus, only male mice were used in the micronucleus assay.

C. Micronucleus assay

GF-871 did not affect the body weight of the animals. There were no indications of systemic toxicity during the in-life portion of the micronucleus test. A summary of the data on the frequencies of MN-PCE and percent PCE observed in various treatment groups of male mice is presented in Table 2. There were no significant differences in MN-PCE frequencies between the groups treated with GF-871 and the negative controls. The adequacy of the experimental conditions for the detection of induced micronuclei was ascertained from the observation of a significant increase in the frequencies of micronucleated polychromatic erythrocytes in the positive control group.

The percent PCE values observed in the GF-871-treated mice were not significantly different from the negative control values. The percent PCE values of the positive control animals were found to be significantly lower than those of the negative control animals.

Table 2: Summary of micronucleated polychromatic erythrocytes (MN-PCE) frequencies and % polychromatic erythrocytes (%PCE), mean±SD

		% MN/CE		Ratio PCE:NCE	
mg/kg bw		this study	historical	this study	historical
GF-871 as XDE-750 TIPA (dosed daily for 2 days)	0	0.7±0.6	0.3-2.2	60.3±7.0	55.8-68.7
	500	1.0±0.4		58.8±4.8	
	1000	0.6±0.6		53.7±9.2	
	2000	1.2±0.9		60.5±4.9	
cyclophosphamide	120	57.3±17.3*	35.9-79.3	48.7±9.1*	42.8-55.0

N = 6 mice/group where 2000 PCE were examined/mouse for MN incidence and expressed as MN/1000 PCE (% MN/PCE); * $p < 0.05$; historical vehicle and positive control data were based on studies conducted from 1998 to 2003; data taken from Table 17, page 40 of Report

III. AUTHORS' CONCLUSIONS:

"*Based upon the results of the study reported herein, it was concluded that the test material, GF-871, did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes when given as a single oral dose to male CD-1 mice. Hence, GF-871 is considered negative in this test system under the experimental conditions used.*"

IV. REVIEWER'S COMMENTS:

The study was properly conducted and the authors' conclusions are acceptable.

005100
(34)

PMRA Primary Reviewer: Brenda MacDonald, DVM **Signature:**
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division **Date** Oct 7, 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509C)
Date 10/20/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat: OPPTS 870.7485; OECD 417.

PC CODE: 005100 **DP BARCODE:** D305670

TEST MATERIAL (PURITY):
i) [^{12}C]XDE-750, purity 99.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid).
ii) [^{14}C]XDE-750, purity 98.6%.

SYNONYMS: Aminopyralid; XR-750; X660750.

CITATION: Jiazhong Liu, Ph.D, et al. (2004)[^{14}C]XDE-750: Absorption, Distribution, Metabolism and Excretion in Male Fischer Rats. ABC Laboratories, Inc., Columbia, Missouri. ABC Laboratories Study No. 47456, The Dow Chemical Study No. 021200, March 12, 2004. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a metabolism study (MRID 46235807), [^{14}C]XDE-750 (radiochemical purity 98.6%), was administered to 4 male Fischer 344 rats/dose as a single gavage dose of 50 or 1000 mg/kg bw, or 14 daily doses (50 mg/kg bw) of unlabelled XDE-750 (purity 99.5%) followed by a single gavage dose of 50 mg/kg bw [^{14}C]XDE-750 on day 15. Excreta were collected at 0, 6 (urine only), 12 (urine only), 24, 48, 72, 96, 120, 144 and 168 hours post-dosing.
Study results indicated that radiolabelled XDE-750 is rapidly absorbed, distributed and excreted following oral administration in rats. Total 24-hour recoveries of the radioactivity were high for all groups (~41-59% and 33-43% of the administered dose in urine and feces, respectively). The absorption and excretion patterns of the ^{14}C moiety were similar among all groups.
Study results indicated that XDE-750 was not metabolized to volatile compounds, including CO_2.
The average α-phase elimination half-lives (T1/2 α) of ^{14}C-XDE-750 equivalents were 2.85, 3.27 and 3.78 hours for the single low, repeated low and single high dose groups, respectively. The average β-phase urinary elimination half-lives (T1/2 β) of ^{14}C-XDE-750 equivalents were 10.23, 12.25 and 10.88 hours for the single low, repeated low and single high dose groups, respectively.
Tissue distribution and bioaccumulation of XDE-750 were minimal; <0.73% of the administered dose was recovered in tissues 7 days after oral administration for all dosing groups. Highest levels of radioactivity were found in the skin and carcass.
XDE-750 was excreted unchanged indicating an absence of metabolism. XDE-750 represented >96% of

the AD in the urine, and 100% of the AD in feces. Three unknown components (≤4%) found in urine were also detected in similar quantities in the analysis of the dose formulation, suggesting that they were trace impurities in the radiolabelled material.

This metabolism study in the rat is classified acceptable and satisfies the guideline requirement for a metabolism study (OPPTS 870.7485); OECD 417 in rats.

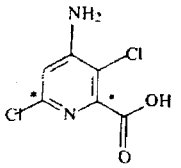
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Compound:

Radiolabelled Test Material:	XD-750-per-2,6-^{14}C
Radiochemical purity	98.6%
Specific Activity	27.4 mCi/mmol
Lot/Batch #:	F380-135a
Non-Radiolabelled Test Material:	^{12}C-XD-750
Description:	Tan powder
Lot/Batch #:	TSN102417
Purity:	99.5% a.i.
Contaminants:	None stated.
CAS #:	150114-71-9



* - denotes position of ^{14}C-label.

2. **Vehicle:** 0.5% methyl cellulose in distilled water (w/w).

3. **Test Animals:**

Species:	Rat, males only.	
Strain:	Fischer 344.	
Age/weight at study initiation:	10 weeks of age; ~200 g to 230 g.	
Source:	Hilltop Lab Animals, Inc., Scottdale, Pennsylvania.	
Housing:	Individually in polycarbonate shoebox cages with hardwood chip bedding. During urine and fecal collection, housed individually in individual glass metabolism cages.	
Diet:	Certified Rodent Diet #5002, *ad libitum*	
Water:	Water provided *ad libitum*, source not stated.	
Environmental conditions:	**Temperature:**	20.19-23.24°C
	Humidity:	42.8-93.8%
	Air changes:	23/hr
	Photoperiod:	12 hrs dark/12 hrs light

Acclimation period: At least one week.

4. Preparation of Dosing Solutions: The respective doses were prepared by mixing ^{12}C-XD-750 and ^{14}C-XD-750 with the appropriate amount of 0.5% methyl cellulose in distilled water (w/w).

B. STUDY DESIGN AND METHODS:

1. Group Arrangements:

Animals were assigned to the test groups noted in Table 1. The method for assignment to the test groups was not described by the study author.

TABLE 1 - Dosing Groups for Pharmacokinetic Studies for XD-750

Test Group	Dose (mg/kg bw)	Actual conc. (mg/kg bw)	Number/sex	Remarks
Single low dose	50	51.1-52.8	4 males	Urine samples were collected at 6, 12 and 24 hours post-dosing, then daily to 168 hours. Feces were collected daily to 168 hours. Volatile organics were collected at 24 hours[a]. Expired CO_2 was collected at 12 and 24 hours[a]. Animals were sacrificed at 168 hours post-dosing, at which time tissues [b] and carcass were retained for analysis.
2-week pretreatment; single oral low dose	50	51.7-52.2	4 males	
Single high dose	1000	1066.9-1231.7	4 males	

[a] Expired CO_2 and volatile organics were collected for 24 hours after the single high dose. Since only 0.01% of the administered dose was detected in the volatiles during the first 24 hours, the collection of volatiles was discontinued for the remaining collection intervals of the single high dose group and also in the single low and multiple low dose groups.

[b] Tissues retained for analysis were perirenal fat, spleen, entire skin, kidneys, liver and gastrointestinal tract (GIT) - including ingesta.

2. Dosing: Each animal was weighed within 4 hours of dosing and the dose required was calculated, dispensed and administered by oral gavage. Dose formulations were warmed to room temperature before dose administration and were stirred constantly on a magnetic plate to ensure homogeneity during dose administration.

Sample Collection: Refer to Table 1, above, for details of urine, feces and tissue sample collection. All urine and fecal samples were collected on dry ice. Samples were bar coded identifying the study number, animal number, sample matrix and collection time or interval. Date of collection and initials of a team member involved in the collection of the samples were added on the container in permanent ink at the time of collection. All samples were weighed at collection.

CO_2 samples were collected using a labeled and tared trap containing 1-methoxy-2-propanol (3:7, v/v). Metabolism cage washes with ~50 mL of deionized water were taken for each 24 hour period post-dosing, and were collected into tared and labeled plastic containers. Final cage wash was performed with ~50 mL of deionized water followed by ~ 50 mL of methanol.

Volatile organic compounds were collected into traps containing polyurethane foam plugs, which were then placed into 50 mL centrifuge tubes.
Whole blood was collected from each animal after sacrifice from the abdominal vein into syringes rinsed with sodium heparin, then transferred into blood tubes containing sodium heparin.
After blood collection, tissues and residual carcass were collected and weighed.

4. Sample Preparation and Analysis - Radiochemical:

i) Urine, carbon dioxide and cage wash: Samples were homogenized before aliquots were taken for LSC. Duplicate aliquots were mixed with ~5 mL (urine) or ~15 mL (CO_2 and cage wash) of Ultima Gold scintillation cocktail in 7- or 20-mL glass scintillation vials, respectively, for LSC.
ii) Feces: a) Fecal samples were homogenized in dry ice or liquid nitrogen using a Waring blender. Duplicate samples were weighed out into 20 mL scintillation vials for solubilization. One mL of sodium hypochlorite solution was added and aliquots were placed in an oven at ~55°C for at least 1 hour. Samples were cooled and then the remaining chlorine was blown out under a stream of nitrogen. Approximately 15 mL of Hionic Fluor scintillation cocktail was added as the scintillant. The solubilization efficiency was then calculated, which was used to adjust the radioactivity in the fecal samples.
b) The 0-24 hour fecal samples from all groups, and the 24-48 hour fecal samples from the single high dose group were also aliquoted in duplicate and combusted in a sample oxidizer to convert the total carbon-14 in the samples to $^{14}CO_2$. $^{14}CO_2$ was trapped using Carbosorb as the trapping agent and Permafluor as the liquid scintillant.
iii) Volatile organic compounds: These were assayed in duplicate aliquots of the methanol extract mixed with ~15 mL of Ultima Gold scintillation cocktail in 20 mL glass scintillation glass vials for LSC. The polyurethane foam plugs were placed in 20 mL glass vials and ~ 10 mL of Ultima Gold scintillation cocktail was added to each vial.
iv) Tissues and residual carcass: Duplicate samples of the solubilized tissues and residual carcass samples were taken. Concentrated HCl was added to neutralize pH and ~15 mL of Hionic Fluor was added to the scintillant.

Radioactivity levels in the various samples were determined by liquid scintillation counting (LSC) using a Beckman Model LS 6000 SC and Model LS 6500 scintillation counters. Disintegrations per minute (dpm) were calculated automatically from counts per minute (cpm) using Beckman's H-number system. Samples with dpm less than the concurrently run background were considered to contain insufficient radioactivity to reliably quantify. The background levels ranged from 21-51 dpm for LSC sample analysis using Ultima Gold as the scintillation fluid, 29-42 dpm for solubilization using Hionic Fluor as the scintillation fluid and 51-61 dpm for combustion using permafluor as the scintillation fluid.

5. Sample Preparation and Analysis - Metabolite Identification:
i) Urine: The majority of the radioactivity (>90%, except for single high dose group which was ~78%) in urine samples was recovered within 24 hours post-dosing.
Composite 0-6 and 6-12 hour urine samples were prepared by combining volumes representing a fixed percentage of the weight of urine samples collected during each time interval, assuming a density of 1 g/mL. Following the pooling of the urine samples, each composite urine sample was diluted with MilliQ water in order to generate a 1 mL HPLC sample of ~100,000 dpm/100 µL.
ii) The majority of the radioactivity (>90%) in fecal samples was recovered within 24 hours (low dose) or 48 hours (high dose) post-dosing. Therefore, the 0-24 hour composite fecal samples of all 3 groups and the 24-48 hour composite fecal sample of the single high dose group were selected for extraction and

HPLC analysis. Composite fecal samples were prepared by combining a fixed percentage of the weight of fecal homogenates within each group, during each time interval. The composite fecal sample was extracted and concentrated prior to HPLC analysis. The sample was vortexed, then centrifuged and the supernatant was transfered to another tube. 1.5 mL of water was added to the fecal pellet and was then vortexed. An additional 1.5 mL water and acetronile was added to the mixture. then vortexed and centrifuged. The supernatant was transferred to another tube and the water/acetronile extraction was repeated. The 3 supernatants were combined and the volume was adjusted to 9 mL with ACN and water (50/50, v/v) and vortexed. Aliquots were analyzed by LSC to determine percent recovery.
The post-extraction solids (PES) were dried under a gentle stream of N_2, and the weights of the dried residues were recorded. Aliquots were solubilized to determine the amount of unextractable radioactivity. Solubilization involved the addition of 1 mL of sodium hypochlorite solution (NaOCl). Samples were sonicated and then placed in an incubator at 50-55°C for 20 min. The samples were prepared for LSC analysis by the addition of 15 mL Hionic Fluor.
A portion of the combined extract underwent further processing by centrifugation and concentration under a gentle stream of N_2. The concentrated extracts were analyzed by LSC and a 50 µL aliquot was subjected to HPLC.
Metabolite characterization was carried out by HPLC and LC-MS/MS analysis.

6. Statistics: Descriptive statistics were used, i.e., mean ± standard deviation. Calculations were made using Microsoft Excel spreadsheets and databases in full precision mode (15 digits of accuracy). Outlying replicates may be tested using Q-test.
The urinary elimination half-lives of ^{14}C-XDE-750 in urine were determined using WinNonlin 4.0.1.

II. RESULTS

A. Pharmacokinetic Studies:

a) Radioactivity Recovery 7 Days Post-Dosing with [^{14}C]XDE-750:
Refer to Tables 2a and 2b.

i) Single Oral Low Dose: Seven days after dosing, the excretion of radioactivity in the urine and feces were 49.83% and 43.05% of the administered dose (AD), respectively. The majority had been excreted within the first 6 hours for urine and 24 hours for feces, i.e., 40.01% (urine) and 41.85% (feces) of the AD.
Tissues and cage wash contained 0.07% and 3.02% of the AD, respectively.
Total recovery of radioactivity was 95.99% of the AD.

ii) Single Oral High Dose: Seven days after dosing, the excretion of radioactivity in the urine and feces were 41.27% and 43.43% of the administered dose (AD), respectively. The majority had been excreted within the first 12 hours for urine and 24 hours for feces, i.e., 32.21% (urine) and 30.53% (feces) of the AD.
Tissues and cage wash contained 0.73% and 9.72% of the AD, respectively.
Total recovery of radioactivity was 95.17% of the AD..

iii) Repeated Oral Low Dose: Seven days after dosing, the excretion of radioactivity in the urine and feces were 58.57% and 32.98% of the administered dose (AD), respectively. The majority had been excreted within the first 6 hours for urine and 24 hours for feces, i.e., 46.68% (urine) and 29.26% (feces)

of the AD.
Tissues and cage wash contained 0.09% and 3.36% of the AD, respectively.
Total recovery of radioactivity was 95.01% of the AD.

TABLE 2a - Cumulative recovery of radioactivity in urine and feces of male rats at various time intervals[a], after dosing with [^{14}C]-XDE-750

Interval	Percent of radioactive dose recovered, cumulative					
	Single oral low dose		Single oral high dose		Repeated oral dose	
	Urine	Feces	Urine	Feces	Urine	Feces
6 hours	40.01	---	20.25	---	46.68	---
12 hours	46.92	---	32.21	---	54.75	---
24 hours	49.08	41.85	36.76	30.53	57.68	29.26
48 hours	49.65	42.85	40.09	40.56	58.29	32.77
72 hours	49.73	42.95	40.90	42.38	58.40	32.85
96 hours	49.76	43.00	41.07	42.91	58.43	32.91
168 hours	49.85	43.05	41.29	43.44	58.57	32.98

[a] Data extracted from pages 21 to 23 of the study repo

TABLE 2b - Recovery of radioactivity in tissues and excreta of rats at 168 hours[a], after dosing with [^{14}C]-XDE-750

	Percent of radioactive dose recovered		
	Single oral low dose	Single oral high dose	Repeated oral low dose
Urine	49.85	41.29	58.57
Feces	43.05	43.44	32.98
Cage wash	3.02	9.72	3.36
Tissues	0.07	0.73	0.09
TOTAL	95.99	95.18	95.00

[a] Data extracted from pages 21 to 23 of the study report

2. Urinary Elimination Half-Lives: The average α-phase elimination half-lives (T1/2 α) of ^{14}C-XDE-750 equivalents were 2.85, 3.27 and 3.78 hours for the single low, repeated low and single high dose groups, respectively. The average β-phase urinary elimination half-lives (T1/2 β) of ^{14}C-XDE-750 equivalents were 10.23, 12.25 and 10.88 hours for the single low, repeated low and single high dose groups, respectively.

3. Tissue Distribution: Refer to Table 3.

i) Single Oral Low Dose: At the end of the seven day post-dosing period, the highest concentrations of [^{14}C]-XDE-750 were found in the skin (0.03% AD; 0.074 µg equiv/g tissue) and remaining carcass (0.04% AD; 0.030 µg equiv/g tissue). Whole blood, liver and kidneys contained 0.026, 0.024 and 0.020 µg equiv/g tissue, respectively, and less than 0.01% of the AD.

ii) Single Oral High Dose: At the end of the seven day post-dosing period, the highest concentrations of [^{14}C]-XDE-750 were found in the skin (0.45% AD; 27.006 µg equiv/g tissue), remaining carcass (0.26% AD; 4.452 µg equiv/g tissue) and GIT with ingesta (0.03% AD; 2.562 µg equiv/g tissue). Whole blood, liver and kidneys contained 0.608, 0.607 and 0.541 µg equiv/g tissue, respectively, and less than 0.01% of the AD.

iii) Repeated Oral Low Dose: At the end of the seven day post-dosing period, the highest concentrations of [^{14}C]-XDE-750 were found in the skin (0.05% AD; 0.148 µg equiv/g tissue) and remaining carcass (0.05% AD; 0.032 µg equiv/g tissue). Whole blood, liver and kidneys contained 0.029, 0.027 and 0.021 µg equiv/g tissue, respectively, and less than 0.01% of the AD.

TABLE 3 - Distribution of radioactivity in male rat tissues/organs after dosing with [^{14}C]-XDE-750[a]

	Single oral low dose		Single oral high dose		Repeated oral low dose	
Tissue/organ	% Dose	µg equiv/g	% Dose	µg equiv/g	% Dose	µg equiv/g
Whole blood	na	0.026	na	0.608	na	0.029
Kidneys	<0.01	0.020	<0.01	0.541	<0.01	0.021
Liver	<0.01	0.024	<0.01	0.607	<0.01	0.027
Perirenal fat	na	0.004	na	0.072	na	0.004
GIT with ingesta	<0.01	0.019	0.03	2.562	<0.01	0.025
Skin	0.03	0.074	0.45	27.006	0.05	0.148
Spleen	<0.01	0.017	<0.01	0.609	<0.01	0.015
Remaining carcass	0.04	0.030	0.26	4.452	0.05	0.032

[a] Data extracted from pages 25 to 27 of the study report

B. Metabolite Characterization Studies:

i) Urine: Refer to Table 4. The metabolic profiles in urine samples of male rats were qualitatively similar across three dose levels and collection intervals. The major portion of the administered dose (AD) of radioactivity was excreted as unchanged XDE-750, accounting for ≥96.0% of the total chromatographed radioactivity. Three unknown components were detected, making up ≤4% of the radioactivity. Since the 3 unknown components were also detected in similar quantities in dose formulations, they were considered most likely to be trace impurities. Hence, further characterization was not performed.

TABLE 4 - Metabolite profile in urine of male rats dosed with [^{14}C]-XDE-750[a], % of administered dose

Dose	Percent of administered dose					
	Single low dose		Single high dose		Repeated low dose	
Compound	0-6 hr	6-12 hr	0-6 hr	6-12 hr	0-6 hr	6-12 hr
XDE-750	38.78	6.76	19.49	11.65	45.58	7.89
U1	0.59	0.07	0.38	0.15	0.53	0.08
U2	0.28	0.03	0.18	0.06	0.29	0.04
U3	0.36	0.04	0.20	0.10	0.28	0.05
Total Identified	38.78	6.76	19.49	11.65	45.58	7.89
Total Radioactivity	**40.01**	**6.91**	**20.25**	**11.96**	**46.68**	**8.07**

* Data extracted from page 31 of the study report

ii) Feces: Refer to Table 5. The metabolic profiles in fecal samples of male rats at all 3 dose levels were characterized by a single peak. The only component observed in feces was the unchanged XDE-750, accounting for 100% of the total chromatographed radioactivity.

TABLE 5 - Metabolite profile in feces of male rats dosed with [^{14}C]-XDE-750*, % of administered dose

Dose	Percent of administered dose			
	Single low dose	Single high dose		Repeated low dose
Compound	0-24 hr	0-24 hr	24-48 hr	0-24 hr
XDE-750	41.85	30.53	10.03	29.26
Total Identified	41.85	30.53	10.03	29.26
Total Radioactivity	**41.85**	**30.53**	**10.03**	**29.26**

* Data extracted from page 35 of the study report

III. DISCUSSION

A. Investigators' Conclusions: "[^{14}C]XDE-750 was readily absorbed and efficiently cleared through urine and feces after oral administration to rats. The absorption and excretion patterns of the ^{14}C moiety were similar among all groups.
Most of the radioactivity was excreted from the rats during the first 24 hours for all dose groups. Approximately 41-59% and 33-43% of radioactivity was recovered in urine and feces, respectively, during 7-day depletion.
Less than 0.1% of the administered dose was detected in the expired air from Group 1 treated animals during the first 24 hour period. It indicated that XD-750 was not metabolized to volatile compounds including CO_2.
Overall recovery of radioactivity was greater than 95% at 7 days after dosing for all dose groups.
XD-750 represented ≥96% of the chromatographed radioactivity detected via HPLC-radiochromatography in the urine. Three unknown components (≤4%) found in urine were also detected

in similar quantities in the analysis of the dose formulation, suggesting that they are trace impurities in the radiolabelled material.
XD-750 represented 100% of the chromatographed radioactivity detected via HPLC-radiochromatography in the fecal extracts.
The PIS (*m/z* 207) analysis of urine sample produced an identical fragmentation pattern compared to a XD-750 reference standard.
The MRM (*m/z* 207/161) analysis of urine and fecal samples confirmed the identity of the major component as the unchanged parent, XD-750.
XD-750 was excreted unchanged indicating an absence of metabolism.
Between 41 and 59% of the administered XD-750 was absorbed and eliminated in the urine of the three dose groups.
It is estimated that <5% of the administered dose absorbed was eliminated in the feces."

B: Reviewer Comments: The reviewer agrees with the study author's conclusions.

C. Study Deficiencies: No scientific deficiencies were noted in the study.

PMRA Sub. No. 2004-0789/DOW AMINOPYRALID/AMD — ~ PROTECTED ~ — Rat Metabolism Study / DACO 4.8

PMRA Primary Reviewer: Brenda MacDonald, DVM
Fungicide/Herbicide Toxicological Evaluation Section, Health Evaluation Division
Signature: [signature]
Date Aug 31 2005

EPA Secondary Reviewer: Karlyn J. Bailey, M.S.
Registration Action Branch 2, Health Effects Division (7509C)
Signature: [signature]
Date 9/1/05

TXR#: 0053657

DATA EVALUATION RECORD

STUDY TYPE: Dissociation and Metabolism in the Rat.

PC CODE: 005100 005209 **DP BARCODE:** D305670

TEST MATERIAL (PURITY):
i) Unlabelled XDE-750, purity 94.5% (3,6-dichloro-4-amino-2-pyridinecarboxylic acid); [^{14}C]XDE-750, purity 98.25%.
ii) Unlabelled XDE-750, triisopropanolamine (TIPA) salt, purity 41.3%; [^{14}C]XDE-750-TIPA, purity 98.25%.

SYNONYMS: i) Aminopyralid; XR-750; X660750.
ii) Aminopyralid triisopropanolammonium; XDE-750-TIPA.

CITATION: Domoradzki, J. Y., et al. (2004) XDE-750, Triisopropanolamine Salt: Dissociation and Metabolism in Male Fischer Rats. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Study No. 031129, February 27, 2004. Unpublished.

SPONSOR: Dow Agrosciences LLC, Indianapolis, Indiana.

PURPOSE: This dissociation and metabolism study (MRID 46235833) was designed to provide additional data on the bioequivalency (in terms of the absorption, distribution, metabolism and elimination) of XDE-750 with that of the triisopropanolammonium (TIPA) salt of XDE-750 following oral gavage administration to rats. This study provides supplemental data on the kinetics and disposition of ^{14}C-XDE-750 and the TIPA salt of ^{14}C-XDE-750 in male Fischer rats. Plasma ^{14}C-kinetics, tissue distribution, excretion and metabolic profiles (via urine and feces) of ^{14}C-XDE-750 and the TIPA salt of ^{14}C-XDE-750 were compared following administration of equimolar oral doses.

EXECUTIVE SUMMARY: In a metabolism study, [^{14}C]XDE-750 (radiochemical purity 98.25%), or [^{14}C]XDE-750-TIPA (radiochemical purity 98.25%) was administered to 4 male Fischer 344 rats/dose as a single gavage dose of 50 or 96 mg/kg bw, respectively. Excreta were collected at 0, 6 (urine only), 12 (urine only), 24, 48, 72, 96 and 120 hours post-dosing. Plasma was prepared from blood collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 120 hours post-dosing.
A single oral administration of ^{14}C-XDE-750 or ^{14}C-XDE-750-TIPA was rapidly absorbed by the rat. The excretion of 38.3% (for ^{14}C-XDE-750) and 34.6% (for ^{14}C-XDE-750-TIPA) of the administered radioactivity in the urine within six hours confirms that the amino-dichloro-picolinate (or anion) portion of the molecule was rapidly absorbed regardless of whether it was administered as the acid or as the

TIPA salt formulation.

Plasma AUCs were 23.0 and 19.0 µg eq-hour/g plasma; half-lives from the α phase of plasma elimination were 0.338 and 0.509 hours; and half-lives from the β phase of plasma elimination were 8.8 and 13.0 hours for the XDE-750 and XDE-750-TIPA dosed groups, respectively. These data indicate that pharmacokinetic behavior was similar between the two compounds.
Based on the amount of radioactivity recovered in the urine through 120 hours, a minimum of 46.3% and 42.5% of the orally administered ^{14}C- XDE-750 and the ^{14}C-XDE-750-TIPA was absorbed. Radioactivity was also rapidly eliminated with 93.5% (44.7% in urine; 48.8% in feces) and 93.3% (41.5% in urine; 51.8% in feces) of the administered doses of ^{14}C-XDE 750 and ^{14}C-XDE-750-TIPA recovered in excreta within 24 hours post-dosing. Urinary rates of elimination calculated for the two compounds were also similar. Half-lives estimated for the rapid initial (α) phase of the urinary elimination curve were 2.8 hours for the ^{14}C-XDE-750 dosed group and 2.5 hours for the ^{14}C-XDE-750-TIPA dosed group. Half-lives estimated for the slower terminal (β) phase were 7.8 hours for the ^{14}C-XDE-750 dosed group and 10.7 hours for the ^{14}C-XDE-750-TIPA dosed group.
The amino-dichloro-picolinate portion of the molecule(s) was excreted primarily unchanged following a single oral administration of either formulation. Parent XDE-750 represented >99% of the radioactivity detected in the urine and feces of both dose groups. The only unidentified metabolite was detected in urine from the ^{14}C-XDE-750-TIPA dosed group, and represented 0.34% of the administered dose.
The results from this study indicate that ^{14}C-XDE-750 and ^{14}C-XDE-750-TIPA, when administered orally to rats, are bioequivalent in terms of absorption, distribution, metabolism, and excretion of the amino-dichloro-picolinate portion of the molecule(s).

This study is classified as acceptable/non-guideline since it was not conducted according to GLP nor according to any specific guideline. It is a special study designed specifically to compare the tissue distribution, excretion and metabolic profiles (via urine and feces) of ^{14}C-XDE-750 and the TIPA salt of ^{14}C-XDE-750. The study is considered acceptable for this purpose.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

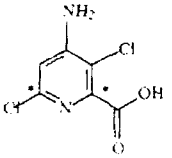
I. MATERIALS AND METHODS

A. MATERIALS:

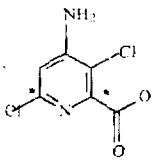
1. Test Compound:

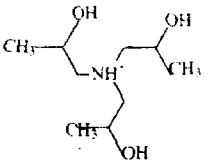
Radiolabelled Test Material:	i) XDE-750-pyr-2,6-^{14}C ii) XDE-750-pyr-2,6-^{14}C, TIPA
Radiochemical purity	i) 98.25% ii) 98.25%.
Specific Activity	i) 28.6 mCi/mmol ii) 28.6 mCi/mmol
Lot/Batch #:	i) SPS reference no. DE3-E1004-77, Inventory No. 1893. ii) SPS reference no. DE3-E1004-77, Inventory No. 1893 and F1063-106.
Non-Radiolabelled Test Material:	i) XDE-750 ii) XDE-750, triisopropanolammunium (TIPA) salt
Description:	Tan powder
Lot/Batch #:	i) TSN102319; Lot# F0031-143. ii) TSN104110; Lot# 173-162-1A.

Purity:	i) 94.5% a.i ii) an aqueous formulation (GF-871) consisting of 41.3% (w/w) as XDE-750, triisopropanolammonium.
Contaminants:	None stated.
CAS #:	i) 150114-71-9; ii) 150114-71-9 and 000122-20-3.
Structures:	i) XDE-750-pyr-2,6-^{14}C



ii) XDE-750-pyr-2,6-^{14}C, TIPA





* - denotes position of ^{14}C-label.

2. **Vehicle:** 0.5% aqueous methyl cellulose.

3. **Test Animals:**

Species:	Rat, males only.	
Strain:	Fischer 344.	
Age/weight at study initiation:	10-11 weeks of age; 173 g to 188 g.	
Source:	Hilltop Lab Animals, Inc., Scottdale, Pennsylvania.	
Housing:	Following dosing, animals were housed individually in glass Roth-type metabolism cages.	
Diet:	Certified Rodent Diet #5002 in pelleted form, *ad libitum*	
Water:	Municipal water, *ad libitum*.	
Environmental conditions:	**Temperature:**	19-24°C
	Humidity:	48-54%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Jugular vein cannulated rats (surgery performed by the supplier) were acclimated in metabolism cages for 2 days prior to dosing.	

4. **Preparation of Dosing Solutions:** The appropriate amounts of unlabelled XDE-750 and ^{14}C-XDE-750, or unlabelled XDE-750-TIPA and ^{14}C-XDE-750-TIPA, were added to 0.5% aqueous methyl cellulose to achieve the desired dose levels.

B. STUDY DESIGN AND METHODS:

1. Group Arrangements:

Animals were assigned to the test groups noted in Table 1. Animals were selected based on the patency

of the jugular vein cannulae and randomly assigned to treatment groups using a computer-driven randomization procedure.

TABLE 1 - Dosing Groups for Pharmacokinetic Studies for XDE-750

Test Group	Dose (mg/kg bw)	Actual conc. (mg/kg bw)	Number/sex	Remarks
^{14}C-XDE-750	50	52.12±1.501	4 males	Urine samples were collected at 6, 12, 24, 36 and 48 hours post-dosing, then daily to 120 hours. Feces were collected daily to 120 hours. Plasma was prepared from blood collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 120 hours post-dosing. Animals were sacrificed at 120 hours post-dosing, at which time tissues [b] and carcass were retained for analysis.
^{14}C-XDE-750-TIPA	96	98.80±3.472	4 males	

[b] Tissues retained for analysis were perirenal fat, spleen, skin, kidneys, liver, gastrointestinal tract (GIT) - including ingesta, residual carcass, plasma (terminal) and whole blood (terminal).

2. **Dosing:** Each animal was weighed just prior to dosing and the dose required (i.e., ~5 g dose solution/kg bw) was calculated, dispensed and administered by oral gavage. Because of the differences in the water solubilities of the 2 test materials, the physical properties of the 2 dose solution formulations were different. XDE-750-TIPA is more water soluble than the acid form of XDE-750 and was completely dissolved in the aqueous-based dosing solution. However, much of the XDE-750 (acid) remained undissolved in the dose solution (i.e., as a suspension). Hence, immediately prior to administration of the gavage dose, the XDE-750 was uniformly resuspended in the dosing formulation (shaking by hand).

Sample Collection and Preparation: Refer to Table 1, above, for details of urine, feces and tissue sample collection. All urine was collected in dry ice cooled traps. Urine traps were changed at the end of each collection interval. At that time, metabolism cages were rinsed with water and the rinse was retained. Each urine specimen and urine/cage wash rinse was weighed, and a weighed aliquot of each sample was analyzed for radioactivity by LSS. Equal volume aliquots of urine samples from the 0-6 and 6-12 hour collection intervals were pooled and stored at -80°C and selected samples from these 2 collection intervals underwent chemical analysis.

Feces were collected in dry ice chilled containers. An aqueous homogenate was prepared, of which weighed aliquots were combusted in a tissue oxidizer and quantitated for radioactivity by LSS. In addition, equal volume aliquots were taken from the 0-24 hour collection interval and pooled. These pooled samples were stored at -80°C and designated for chemical analysis.

At 120 hours post-dosing, animals were anesthetized with a CO_2/O_2 mixture and sacrificed by exsanguination, and the appropriate tissues were collected. The carcass, GIT with contents, kidneys, liver and whole blood were homogenized and a weighed aliquot oxidized and analyzed for radioactivity by LSS. A representative skin sample was directly oxidized and analyzed for radioactivity by LSS. Perirenal fat and the spleen were directly oxidized without homogenization.

Final cage wash and contents were collected, weighed and analyzed for radioactivity.

Except for the terminal blood sample (of which a portion was used for plasma), blood samples were centrifuged to obtain plasma which was analyzed for radioactivity to constrict the plasma ^{14}C-concentration-time courses.

Control urine and feces were collected in dry ice traps from 1 undosed male rat. Control excreta was collected for a 48-hour period, beginning on the day that the treated animals were dosed. The control rat was sacrificed at study termination and blood was collected by the same procedure as the treated animals.

Tissues were not collected.

4. **Sample Analysis - Radiochemical:** Radioactivity levels in the various samples were determined by liquid scintillation counting (LSC) using a Packard TriCarb 2900TR. Counts per minute (cpm) were corrected for quench and background, and converted to disintegrations per minute (dpm). Samples with dpm less than twice the concurrently run background (blanks) were considered to contain insufficient radioactivity to reliably quantify.

5. **Sample Preparation and Analysis - Metabolite Identification:** Final extracts of selected, pooled fecal homogenates were prepared immediately prior to analysis and those extracts, as well as the pooled 0-6 hour urine samples were analyzed in duplicate by HPLC with in-line radiochemical detection to obtain chromatographic radioprofiles of parent compound and possible metabolites.
Urine collected after the first collection interval (0-6 hours) was not radioprofiled since the ^{14}C activity in the urine from the first collection interval accounted for 83% and 81% of the total ^{14}C activity excreted in the urine across all sampling intervals for XDE-750 and XDE-750-TIPA, respectively. Similarly, feces collected after the first collection interval (0-24 hours) was not radioprofiled since the ^{14}C activity in the feces from the first collection interval accounted for 96% and 95% of the total ^{14}C activity excreted in the feces across all sampling intervals for XDE-750 and XDE-750-TIPA, respectively.
Following the chromatographic profiling, representative samples were also analyzed by liquid chromatography/mass spectrometry (LC/MS) in an attempt to confirm the identity of the major Radiolabelled peak as parent compound and to identify those metabolites that exceeded 5% of the administered dose in the urine and fecal specimens.

6. **Statistics:** Descriptive statistics were used, i.e., mean ± standard deviation. Calculations were made using Microsoft Excel spreadsheets and databases in full precision mode (15 digits of accuracy). Pharmacokinetic parameters were determined, including AUC (area-under-the-curve) and elimination rate constants, using a spreadsheet-based (Microsoft Excel) pharmacokinetic computer modeling application. Additional statistical analyses were not performed (not warranted) since a direct comparison of the ^{14}C plasma concentration-time curves and the pharmacokinetic parameters derived from those curves together with comparisons of the rates and extent of ^{14}C excretion in urine and feces were sufficient to demonstrate the bioequivalence of XDE-750 and the TIPA salt of XDE-750.

II. RESULTS

A. Pharmacokinetic Studies:

a) Radioactivity Recovery 5 Days Post-Dosing with [^{14}C]XDE-750 or [^{14}C]XDE-750TIPA:
Refer to Table 2.

i) [^{14}C]XDE-750: Five days after dosing, the excretion of radioactivity in the urine and feces were 40.12% and 50.56% of the administered dose (AD), respectively. The majority had been excreted within the first 6 hours for urine and 24 hours for feces, i.e., 34.08% (urine) and 48.79% (feces) of the AD.
Cage wash contained 6.38% of the AD.
Plasma and tissues contained 0.05% and 0.01% of the AD, respectively.
Total recovery of radioactivity was 97.06% of the AD.

ii) [^{14}C]XDE-750-TIPA: Five days after dosing, the excretion of radioactivity in the urine and feces were 36.87% and 54.30% of the administered dose (AD), respectively. The majority had been excreted

within the first 12 hours for urine and 24 hours for feces, i.e., 30.16% (urine) and 51.76% (feces) of the AD.
Cage wash contained 5.74% of the AD.
Plasma contained 0.05% of the AD; there was no detectable radioactivity recovered from tissues.
Total recovery of radioactivity was 96.91% of the AD.

TABLE 2 - Cumulative recovery of radioactivity in urine and feces of male rats at various time intervals. after dosing with [^{14}C]-XDE-750 or [^{14}C]-XDE-750-TIPA[a]

Interval	Percent of radioactive dose recovered, cumulative					
	[^{14}C]-XDE-750, 50 mg/kg bw			[^{14}C]-XDE-750-TIPA, 96 mg/kg bw		
	Urine	Cage wash	Feces	Urine	Cage wash	Feces
6 hours	34.08	4.26	---	30.16	4.40	---
12 hours	37.18	9.28	---	34.18	4.92	---
24 hours	39.00	5.67	48.79	36.26	5.28	51.76
48 hours	39.97	6.08	50.33	36.73	5.51	54.05
72 hours	40.06	6.12	50.48	36.80	5.55	54.19
120 hours	40.12	6.38	50.56	36.87	5.74	54.30

[a] Data extracted from pages 38 and 39 of the study report

2. **Concentration-Time Course of Radioactivity in Plasma**: Refer to Table 3. The highest concentration of radioactivity was observed in the first plasma samples (0.25 h) from both dose groups. The concentration of ^{14}C activity in plasma decreased bi-exponentially for both dose groups over the collection period. Approximately a 10-fold decrease in plasma concentrations occurred during the first 2 hours post-dosing. By 48 and 24 hours post-dosing, plasma radioactivity levels were non-quantifiable for the ^{14}C-XDE-750 and ^{14}C-XDE-750-TIPA groups, respectively. Plasma elimination half-lives estimated for the rapid initial (α) and slower terminal (β) phases of the curve were 0.3 and 8.8 hours for the ^{14}C-XDE-750 group, and 0.5 and 13.0 hours for the ^{14}C-XDE-750-TIPA group. The 12-hour area-under-the-plasma-curves (AUC's) were calculated as 23.0 and 19.0 µg eq-hour/g plasma for the ^{14}C-XDE-750 and ^{14}C-XDE-750-TIPA groups, respectively. Based on these data, it appears that absorption of both test materials following oral administration was very rapid, with the highest concentration of radioactivity in plasma (C_{max}) occurring before the first plasma samples were taken. Because of the rapid absorption, there are essentially no concentration-time values defining the absorptive phase of the plasma time curve and the pharmacokinetic parameters which would define the absorptive phase (k_a, actual C_{max}, actual T_{max}) cannot be calculated with a high degree of accuracy. However, since plasma concentrations of radioactivity decreased with each successive time point, it is reasonable to state that the actual T_{max} was less than 0.25 hours.

TABLE 3 - Concentration of radioactivity in plasma of male rats at various time intervals, after dosing with [^{14}C]-XDE-750 or [^{14}C]-XDE-750-TIPA[a]

Interval	Concentration of Radioactivity in Plasma, µg-eq/g plasma	
	[^{14}C]-XDE-750, 50 mg/kg bw	[^{14}C]-XDE-750-TIPA[b], 96 mg/kg bw
0.25 hours	25.834	15.522
0.5 hours	17.013	13.977
1 hour	7.830	7.653
2 hours	1.162	1.805
4 hours	0.357	0.385
6 hours	0.308	0.296
8 hours	0.308	NA
10 hours	0.317	0.259
12 hours	0.217	0.218
24 hours	0.052	NQ (0.057)
48 hours	NQ (0.050)	NQ (0.030)
120 hours	NQ (0.025)	NQ (0.026)

[a] Data extracted from pages 40 and 41 of the study report.
[b] Plasma concentrations converted from µg-eq ^{14}C-XDE-750-TIPA/g plasma to µg ^{14}C-XDE-750 acid equivalent/g plasma.
NQ - Non-quantifiable
NA - Not applicable

B. Metabolite Characterization Studies:

i) [^{14}C]-XDE-750: Refer to Table 4. The only component observed in urine and fecal samples was unchanged XDE-750, accounting for 46.28% and 50.56% of the administered dose (AD) in urine and feces, respectively. Total recovery of radioactivity was 96.85% of the AD.

TABLE 4 - Metabolite profile in excreta of male rats dosed with [^{14}C]-XDE-750 or ^{14}C]-XDE-750-TIPA, % of administered dose

Dose	Percent of administered dose			
	[^{14}C]-XDE-750		[^{14}C]-XDE-750-TIPA	
Compound	Urine	Feces	Urine	Feces
XDE-750	46.28	50.56	42.12	54.29
U1	---	---	0.34	---
U2	---	---	---	---
U3	---	---	---	---
Total Identified	46.28	50.56	42.12	54.29
Total Radioactivity	46.28	50.56	42.46	54.29

[a] Data extracted from page 31 of the study report

ii) Feces: Refer to Table 4. The major portion of the administered dose (AD) of radioactivity in the excreta was excreted as unchanged XDE-750, accounting for 42.12% and 54.29% of the AD in urine and feces, respectively. One unknown component was detected in urine only, making up 0.34% of the radioactivity. Total recovery of radioactivity was 96.75% of the AD.

III. DISCUSSION

A. Investigators' Conclusions: "A single oral administration of ^{14}C-XDE-750 or ^{14}C-XDE-750-TIPA was rapidly absorbed by the rat. The rapid absorption was evident from the observation that the highest concentration of radioactivity in the plasma was found in the blood samples collected at 0.25 hour post-dosing for both compounds. The excretion of 38.3% (for ^{14}C-XDE-750) and 34.6% (for ^{14}C-XDE-750-TIPA) of the administered radioactivity in the urine within six hours confirms that the amino-dichloro-picolinate (or anion) portion of the molecule was rapidly absorbed regardless of whether it was administered as the acid or as the TIPA salt formulation. Similar pharmacokinetic behavior between the two compounds was also evident based on PK parameters derived from the 0 to 12-hour portion of the plasma concentration-time curves. The following pharmacokinetic parameters were derived for the XDE-750 and XDE-750-TIPA dosed groups, respectively: plasma AUCs were 23.0 and 19.0 µg eq-hour/g plasma; half-lives from the α phase of plasma elimination were 0.338 and 0.509 hours; and half-lives from the β phase of plasma elimination were 8.8 and 13.0 hours. Based on the amount of radioactivity recovered in the urine through 120 hours, a minimum of 46.3% and 42.5% of the orally administered ^{14}C-XDE-750 and the ^{14}C-XDE-750-TIPA was absorbed. In addition to being rapidly absorbed, the radioactivity associated with the two compounds were rapidly eliminated with 93.5% (44.7% in urine; 48.8% in feces) and 93.3% (41.5% in urine; 51.8% in feces) of the administered doses of ^{14}C-XDE 750 and ^{14}C-XDE-750-TIPA recovered in excreta within 24 hours post-dosing. Urinary rates of elimination calculated for the two compounds were also similar. Half-lives estimated for the rapid initial (α) phase of the urinary elimination curve were 2.8 hours for the ^{14}C-XDE-750 dosed group and 2.5 hours for the ^{14}C-XDE-750-TIPA dosed group. Half-lives estimated for the slower terminal (β) phase were 7.8 hours for the ^{14}C-XDE-750 dosed group and 10.7 hours for the ^{14}C-XDE-750-TIPA dosed group. HPLC radioprofiling (with MS confirmation) of pooled urine and fecal extracts indicated that the amino-dichloro-picolinate portion of the molecule(s) was excreted primarily unchanged following a single oral

administration of either formulation. Except for a minor radioactive peak detected in the 0 to 6-hour pooled urine sample from the ^{14}C-XDE-750-TIPA dosed group (accounting for 0.34% of the administered dose), the only radiolabelled peak detected in analyses of urine or fecal extracts was confirmed as parent XDE-750 (based on retention time and mass spectral matches with an authentic standard of XDE-750). The results from this study indicate that ^{14}C-XDE-750 and ^{14}C-XDE-750-TIPA, when administered orally to rats, are bioequivalent in terms of absorption, distribution, metabolism, and excretion of the amino-dichloro-picolinate portion of the molecule(s)."

B: Reviewer Comments: The reviewer agrees with the study author's conclusions.

C. **Study Deficiencies:** No scientific deficiencies were noted in the study.

13544

R124355

Chemical: Aminopyralid

PC Code: 005100
HED File Code: 13000 Tox Reviews
Memo Date: 11/22/2005
File ID: TX0053657
Accession #: 412-06-0013

HED Records Reference Center
3/30/2006